WORLD
# animal
REVIEW
*a quarterly journal on animal health, production and products*
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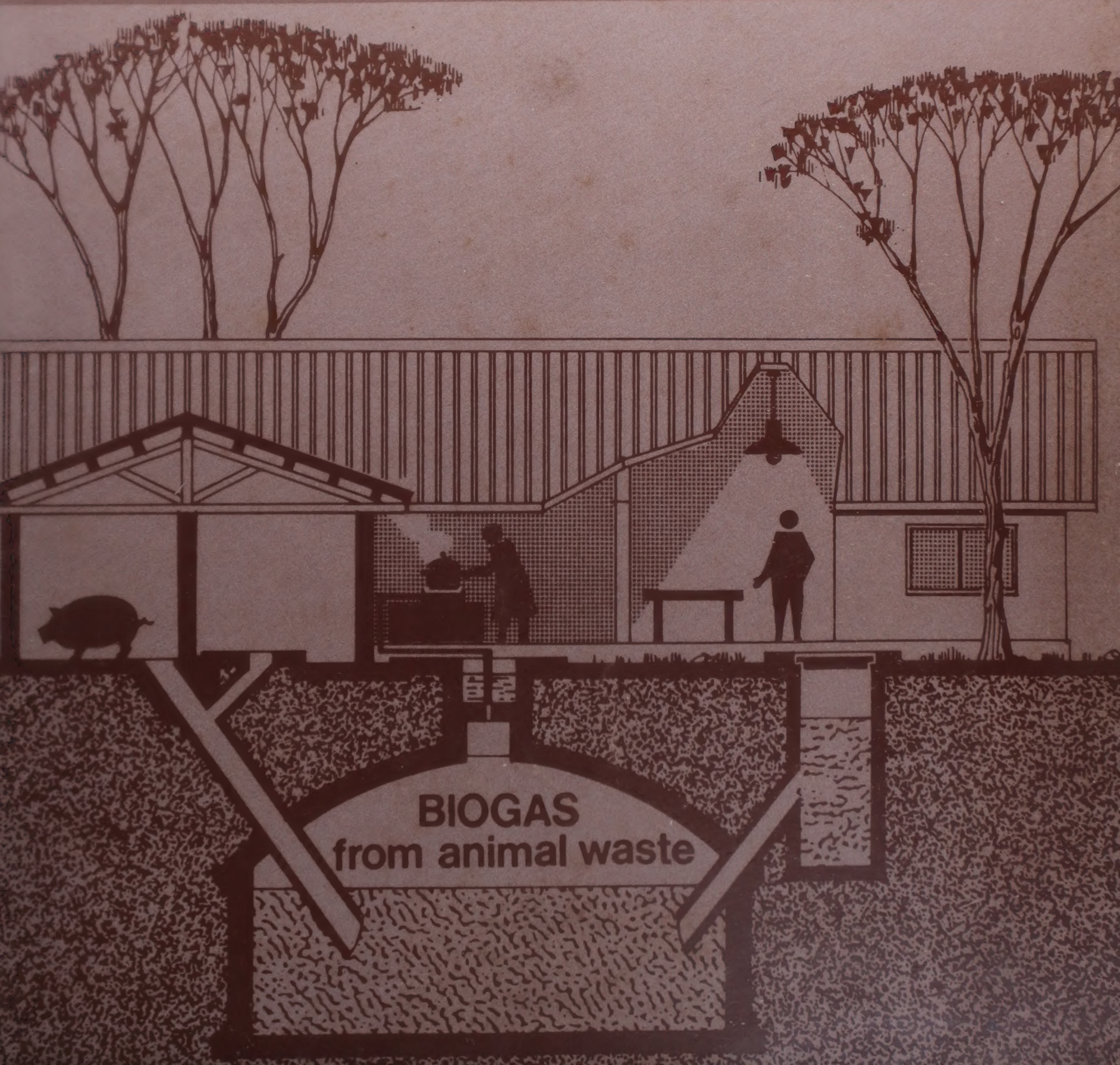

# Energy from animals

In the previous issue of this journal attention was focused on the increasing importance that, in developing countries, was likely to be attached to animals for draught power in agricultural production systems and for load carrying in non-agricultural activities.

Another aspect of the value and importance of animals, apart from their capacity to produce meat, milk and by-products and their use for draught power, is the contribution they can make, especially in developing countries, toward meeting the world's future energy needs.

Estimates have indicated that animal dung, agricultural wastes and wood account for up to 90 percent of the total energy needs in the rural sectors of developing countries (other than OPEC countries). Finding substitutes for these becomes increasingly difficult while total dependence on them has resulted, in some areas, in depleted forests, soil run-off and erosion, desertification and decreasing crop yields. Farmers in developing countries are often so poor that they cannot afford costly fuels and fertilizers, and their food supplies can well be threatened.

For the promotion of development and the improvement of living standards, especially in the rural areas of developing countries, increasing the use of energy and using it more efficiently are of vital importance. Organic matter in the form of manure has been used for centuries to maintain or increase soil productivity and the terms "manure" and "fertilizer" have been practically synonymous. Because of the shortage of wood fuel in many countries, sun-dried dung has had to be used as a fuel so that its value as a fertilizer has been lost.

Agricultural improvement and increased food production depend in many tropical countries on organic residues as fertilizer because tropical soils are often lacking in humus and commercial fertilizers are frequently beyond the reach of farmers. In producing biogas from animal manure and farm wastes it is possible to obtain energy without much loss of their fertilizer value and this process, therefore, could make an important contribution toward solving the problems of energy and fertilizer.

The direct digestion of organic residues (collectively known as "biomass" — a word in increasing use) such as animal manure by bacteria produces a combustible gas — methane. The residues from this process can be returned to the land to maintain soil condition and improve fertility. For these reasons anaerobic fermentation has been of interest to many countries and, using mainly animal manure, the system has been applied successfully in a number of countries such as China, India, the Republic of Korea, Thailand and other Southeast Asian countries, and, to a limited extent, in Tanzania in Africa.

Biogas not only provides a source of heat for cooking and a source of light but can be used in internal combustion engines for work. Furthermore, it can contribute very usefully toward solving the problems of farm waste disposal and the sanitary disposal of human wastes.

It is, of course, true that digesters are relatively costly and villages in rural areas and individual families will need government help to develop such a facility. The relatively heavy investment involved has not, however, stopped the construction of several million digestion plants in the Sichuan province of China alone.

In this issue of *World Animal Review* the first part of an important article entitled "Biogas — energy recovery from animal wastes" will be found. This first part covers in some detail the principles governing the anaerobic process of biogas production while the second part, in the following issue, will include descriptions of digester systems in use in a number of developed and developing countries and a discussion of the economic aspects of biogas plant operations.

The article reaches the conclusion that methane gas production from animal wastes can be a significant source of energy at local or individual farm level in the rural sector even if, at the national level, biogas plants can have relatively little effect in meeting overall national needs.

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COVER: Home uses of biogas from animal wastes.

# BIOGAS

## energy recovery from animal wastes

part I

**E.P. Taiganides**

*The increasing importance of the world energy problem makes this article on biogas of particular interest now. It has been necessary to divide the article into two parts. Part I in this issue will deal with the principles governing the anaerobic process involved in biogas production and with the design, operation and monitoring of the digester plant. Part II, in the following issue, will include descriptions of actual digester systems which have been operating over the last decade or so in India, Federal Republic of Germany, China and USA and will conclude with a review of the economic aspects of biogas plant operations.*

### Why biogas?

The term "biogas (or bio-gas) plant" normally refers to tanks built to produce methane gas through the anaerobic digestion of farm wastes and other biomass, such as manures, night soil and crop residues. Biogas plants in city sewage-treatment plants are called "sludge digesters". Other names for biogas are "bihugas" in the Federal Republic of Germany, "gobar gas" in India, "marsh gas" in China and some other regions. It was the discovery, some 200 years ago, that gases emitted from water-inundated marshlands were combustible that led to the theory and current practice of gas energy recovery from the decomposition of organic materials in a liquid but oxygen-free environment.

During the last decade, energy costs began to spiral out of control and cast doubt on the dependability of uninterrupted supplies of energy from traditional sources. These developments, along with increased public awareness of the potential of biological processes in converting biomass to energy, are ushering in an era of intensive scientific scrutiny of non-conventional sources of energy in particular, and of resource conservation in general. The extraction of biogas from waste has captured the imagination of people and, as a result, some who equate statistics on the "potential" of the process with "off-the-shelf technology" have been lulled into believing that launching a campaign to popularize biogas plants is all that is needed not only to solve energy supply problems on farms but also the animal-waste problem itself.

### Myths about biogas

Before proceeding any further, some of the myths about biogas need to be dispelled:

First, *the biogas plant is the panacea we have been looking for to solve our animal-waste problem!* False. Neither biogas, nor any other treatment method, can ever be a panacea. This is because of the infinite variability of conditions on animal farms and their different requirements. Energy recovery is applicable only on farms where gas energy can readily be utilized and on farms where there are available management skills, or a tradition of operating biogas plants. The gas energy needs must be documented not only in terms of their quantity but also in terms of their seasonal variation. The best conditions for the introduction of biogas occur when there is a steady demand for the energy produced so that a plant is operated with minimum gas storage facilities. Biogas cannot

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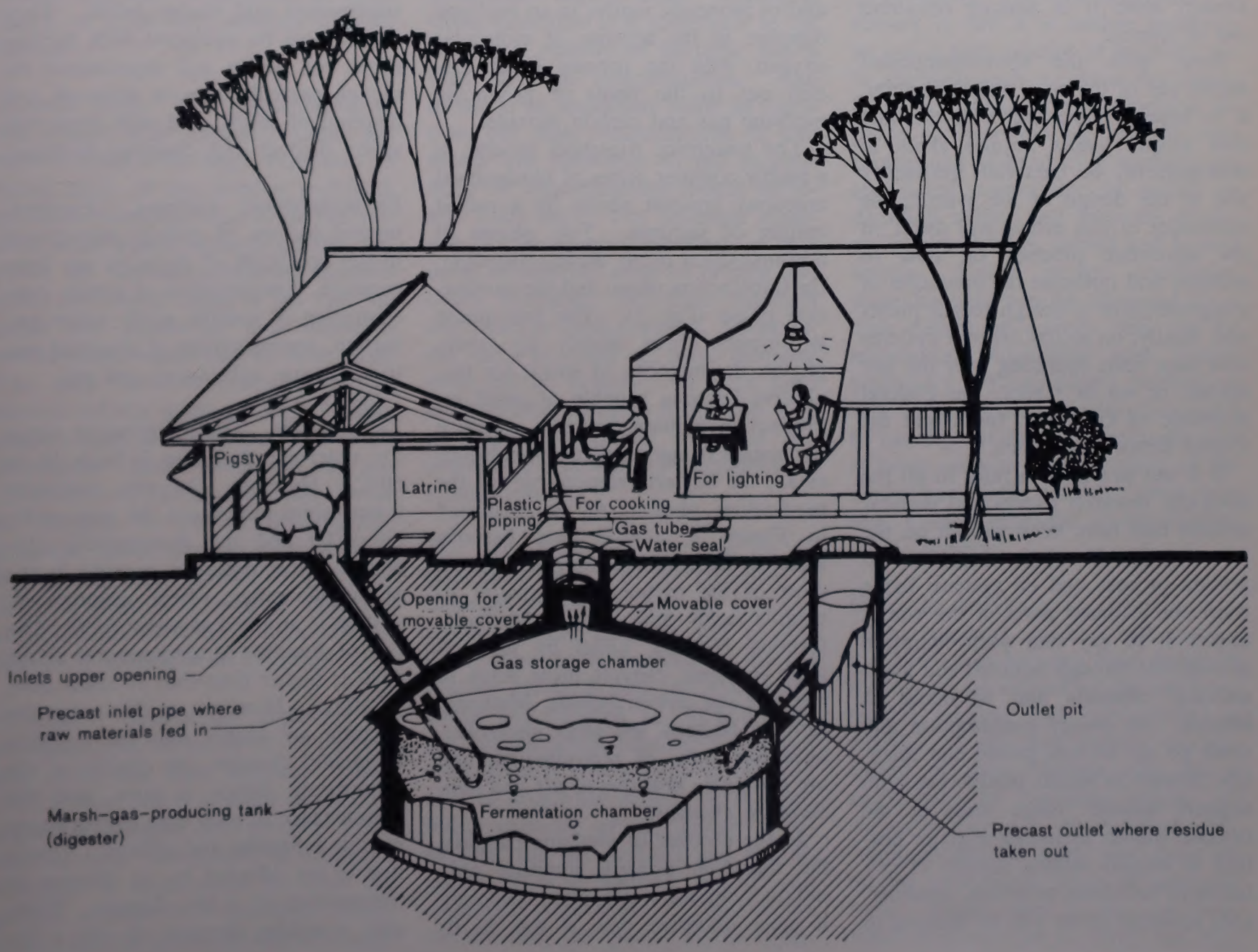
compete in places where piped or bottled gas is readily available, even if the latter costs a great deal more than biogas.

Second, *biogas reduces very greatly the volume of wastes to be handled!* False. Only the organic matter of the solids is reduced by 50 to 70 percent. Since organic content is in the range of 2 to 5 percent of the total waste, even a 70 percent reduction of it would not affect the total waste volume by much. What is reduced is the offensiveness of the raw waste through the stabilization of the biodegradable organic matter, which also reduces the pollution potential of the waste. In addition, most pathogenic bacteria are eliminated if the detention time in the digester is long enough. Another great advantage, especially with wastes from grass-fed animals, is the destruction of weed seeds during digestion.

Third, *it is a simple fermentation process by which methane bacteria turn waste into a combustible biogas!* False. It is a highly complex series of reactions brought about by a mixed culture of bacteria. There are two essential groups of bacteria. The first group liquefy and convert large-molecule organic compounds into small-molecule acid compounds. The second group ferment the acids into methane. Both groups are found universally in nature, particularly in manures, so that pure cultures are not needed to initiate the process. All that is needed is proper management of the environment, i.e., a steady temperature (35°C), oxygen-free atmosphere, steady, constant feeding (in the range of 1 to 6 kg of volatile solids (TVS) per $m^3$ of digester volume), a detention time of 8 to 60 days (only a few days if mixing of digester contents is undertaken) and a low concentration of heavy metals. Once proper growth conditions are established, the bacteria can be activated to a high degree and this can be maintained indefinitely provided the environment is kept favourable. Any sudden changes in environmental factors result in severe disruption of the process and even "murder" of the digester, i.e., the acid-production rate is much higher than its conversion to methane and the digester goes "sour" or "dead". Then foul odours are given off, and it may take several weeks to months to get the digester restarted. This is the reason

*A simple biogas system*



why good management skills are essential even for small digesters.

Fourth, *biogas is produced, but nutrients are lost!* False. Quite the contrary, in fact: digestion enhances the value of the waste as a source of plant nutrients. Biogas is only carbon and hydrogen. Thus all the nitrogen, phosphorus, potassium and the other minerals remain in the waste. Furthermore, since some mineralization occurs, the fertilizer value of the residue solids is greater than in the raw waste. The end-products, besides biogas, are inoffensive in odour, soupy in texture, are free flowing, shed water more easily than raw waste and can thus be dried more readily than fresh waste.

Fifth, *biogas makes economic sense because of the recent hikes in energy costs!* False. Digestion is more of a conservation scheme than an economic investment. Energy and nutrients are conserved instead of wasted. Digestion does not have to make money, since it is *making resources out of wastes!*

Now, with the above-mentioned myths out of the way, and the reader, it is hoped, impressed with the fact that biogas plants require skills in management, in bacterial husbandry and in the design of the system, the remainder of this article will dwell on the anaerobic process, on how to monitor and optimize the operation of a digester, how to design biogas plants and, finally, on actual digester systems that have been operating over the last decade or so in China, the Federal Republic of Germany, India and the United States of America.

It is not practical to refer to all the booklets, research papers and popular articles that have been written on the potential benefits of the anaerobic digestion process and on systems being tried out in many parts of the world. Suffice it to say that such literature is available through national and international channels and in many libraries. As energy consumption and costs go up, so will public and scientific interest in biogas production from organic wastes. Since most of the organic wastes, including a major portion of animal wastes, consist of cellulose or cellulosic materials, considerable scientific effort will be directed to the fermentation of such material, the potential of which is even greater than that of animal wastes. Scientific studies of organic-matter fermentations have been going on for the past 200 years, but design and operation of methane-producing digesters started only about 50 years ago (Tietjen, 1975). Only during the last decade has there been an organized exchange of information on design and construction of biogas plants. As a result of this exchange of knowledge, and of associated advances in technology and their transfer, improvements in biogas production should be forthcoming.

## The digestion process

ANAEROBIC PROCESSES. Treatments that operate in the absence of oxygen are referred to as anaerobic processes. Of these, anaerobic digestion is a treatment designed to decompose organic and/or inorganic matter in an enclosed digester, in the absence of molecular oxygen, with the process being carried out to the point of producing methane gas and carbon dioxide.

The anaerobic digestion process is a highly complex series of biochemical reactions brought about by a mixed culture of bacteria. Two phases of decomposition occur during digestion: the liquefaction phase and the gasification phase (Fig. 1). The first phase is brought about mainly by saprophytes, the majority of which are facultative bacteria capable of rapid reproduction without being as sensitive to changes in environmental conditions as are the bacteria responsible for the gasification phase. As is illustrated in Figure 1, acid-forming bacteria aided by extracellular enzymes convert most of the carbonaceous matter to volatile acids and water. Methane-forming bacteria, aided by intracellular enzymes, convert these acids to methane and carbon dioxide. Methane-forming bacteria are *strict anaerobes*, have *low rates of reproduction*, and are *extremely sensitive to pH and temperature changes.*

In the absence of methane bacteria, the digestion process succeeds only in liquefying the waste, often rendering it more offensive than it was in its original state. However, if, under certain conditions, liquefaction proceeds at a faster rate than gasification, the resultant accumulation of acids inhibits further the methane bacteria, and the digestion process malfunctions. Thus, both types of bacteria must be in proper balance. However, the optimum conditions for the gasifying bacteria are also satisfactory for the liquefying bacteria.

The liquefied waste in the digester is called *supernatant*, while the stabilized solids are called *digested sludge.* Both of these materials must be removed from the digester at regular intervals in order to prevent inhibition of the anaerobic process. The anaerobic digestion process takes place in an airtight tank in which environmental factors are controlled and space is provided for the solids, liquids and gases that are generated. Well-designed digesters should have sampling pipes at least 75 mm in diameter for both supernatant and sludge solids. They should also be equipped with mechanisms for sludge and supernatant removal, gas collection or draw-off and disposal of solids, and with explosion safety devices and drainage facilities.

ENVIRONMENTAL FACTORS. Environmental factors of critical importance in the operation of digesters are temperature, concentration of solids, concentration of volatile acids, scum formation, concentration of essential nutrients, toxic substances and pH.

*Temperature.* The temperature range for anaerobic digestion is from 10 to 60°C. However, the two optimum temperature ranges are the *mesophilic* (30-40°C) and the *thermophilic* (45-60°C). Most digesters operate in the mesophilic range, where optimum digestion is attained at about 35°C. The rate of digestion at temperatures above 45°C is higher than that at lower temperatures. However, in this range the bacteria are highly sensitive to environmental changes, the quality of the supernatant liquor is poor, and the maintenance of such high temperatures is both expensive and difficult. Digestion is not affected by an increase in temperature of a few degrees. However, a sudden decrease of only a few

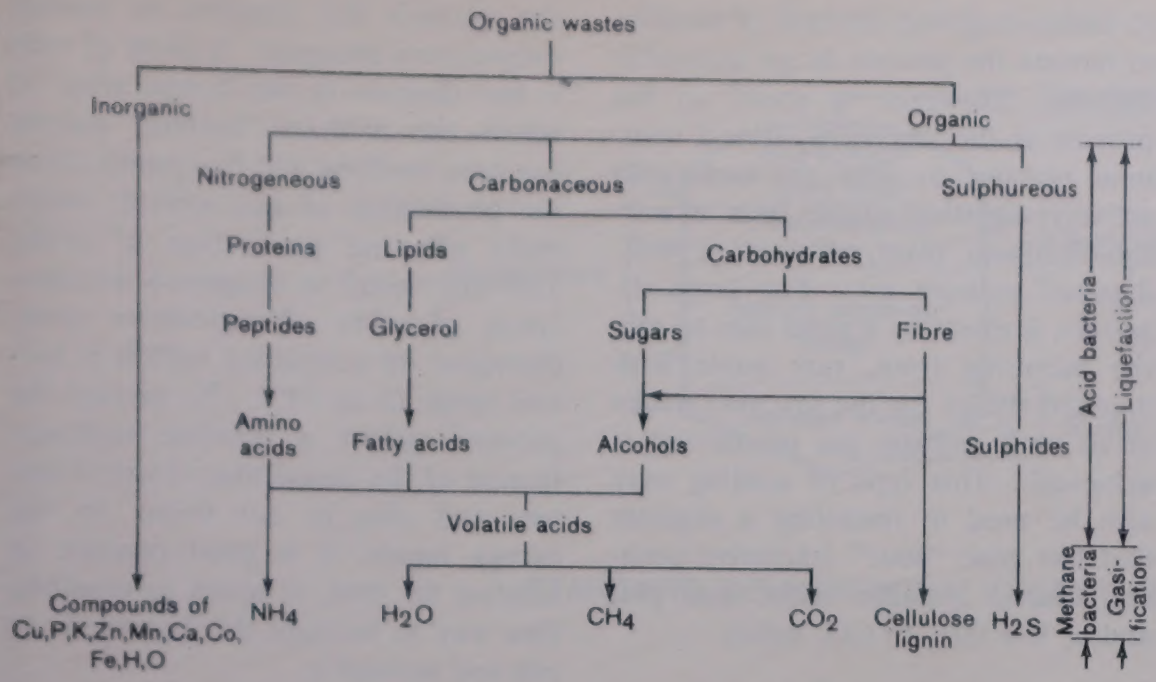



**Figure 1.** *The main paths of the anaerobic digestion process*

degrees could arrest the production of methane without materially affecting the acid-producing bacteria; this would then lead to excessive accumulation of acids and possibly digester failure.

*Volatile acids.* The concentration of the volatile acids normally should not exceed 2 000-3 000 mg/l, expressed as acetic acid. If this level is exceeded, methane formation may drop, the volatile acids would increase rapidly, because the methane-producing bacteria could not utilize them at the rate the acids are produced, and digestion would cease within two to three days. The main causes of excessive production of volatile acids are high loading rates, a drop in temperature and scum formation. Scum formation and accumulation of fibrous materials should be avoided by mixing the digester contents, which also aids the process by establishing uniform conditions. Scum constitutes a zone of substrate accumulation in which a high concentration of volatile acids develops.

*Minerals.* Satisfactory digestion at concentrations of solids of 15 percent and above has been attained. However, a more practical range of concentration of solids is 3-10 percent. Presence of toxic substances in excessive amounts is detrimental in that the growth of bacteria is inhibited. Antibiotics used in animal production units find their way into wastes, but their effect on digestion has not been ascertained. It is not expected to be significant under normal usage. Copper in small quantities is a stimulator to the enzymatic activity of bacteria, but is toxic in high concentrations. Ammonia is an important buffer in digestion, but high concentrations of ammonia are inhibitory to the process. $NH_3$—N concentrations should be below 2 000 mg/l. For optimum digestion, all the essential elements for bacterial metabolism must be present and easily available. All these elements are found in feedlot wastes. However, the ratio of some of these elements must be in balance. A C:N ratio (carbon and nitrogen) of around 16:1 is considered optimal for good gas production and stable fermentation of animal wastes.

*pH.* The optimum pH range for digestion is from 7 to 8. Digestion is inhibited below a pH of 6.5 and ceases entirely below a pH of 4.5. Once digestion is established, the pH reaches about 7, and the sludge becomes well buffered, i.e., its hydrogen-ion concentration remains unchanged even when relatively large amounts of acid or alkali are added. If the buffering capacity of the sludge is destroyed, the pH declines and the digester ceases to function properly and eventually goes "sour", i.e., foul odours are released, the quality of the supernatant and of the sludge solids becomes poor, and methane gas is not produced. It might take days, or even months, to bring a digester that has gone "sour" back into operation.

## Digester design and operating conditions

Optimum operating conditions depend on the primary objective of digestion. If high gas recovery and high solids treatment are essential, then digester temperature, loading rate, detention time and mixing rate must be controlled. In small biogas plants, where nutrient conservation is important, the control of all these factors is not so vital. In either case, the digestion process is self-initiated once anaerobic conditions are established.

The biogas digestion process differs in many respects from other types of fermentation. The most important difference is that it is neither necessary to use a pure culture of bacteria nor to maintain such a culture for inoculation and re-inoculation. Bacteria capable of decomposing organic substances and of producing methane gas are found universally in nature in decaying matter and they are particularly abundant in animal and human wastes. Under proper conditions, these bacteria can be activated to a high degree of activity that can be maintained indefinitely with proper husbandry. Thus, biogas production was not invented or really discovered by anyone. It happened naturally and is being used in different parts of the world but, until recently, there was only a scant exchange of information on the subject.

DIGESTER COMPONENTS. Digesters may be of standard- or high-rate design, the differences between them being in the degree of control of the environmental factors, and particularly in whether or not they employ mixing. Either of these designs may be a single-stage or two-stage system, as shown in Figure 2. In standard, conventional digester tanks that have no mixing mechanism, layers of materials form. Biogas rises to the top from which it is removed for direct use (this

is the least expensive and least cumbersome method), or for temporary storage and/or cleaning. A scum layer is formed by floating matter that rises to the liquid surface with the gas bubbles. Unless the scum layer is disturbed periodically, it can present a serious operational problem in animal-waste digester tanks. Below the scum layer is supernatant liquid that is relatively clear, containing mainly dissolved solids and colloidal materials. Most of the suspended solids, including bacteria, are found in the layer below, where active digestion takes place. Solids, the organic matter of which has been digested, sink to the bottom of the tank. As is shown in Figure 2, waste should be pumped in at various heights in order to provide some mixing in digester tanks without mixers. Supernatant is drawn off from the top of the tank, while digested sludge is extracted from the tank bottom.

In two-stage high-rate (complete mixing of tank contents) digesters, the second tank acts more as a storage and solids-stabilization tank, while the first tank is designed to provide optimum conditions for gasification. Figure 2 also shows all the components of a single-stage high-rate digester. High-rate digesting is carried out in large tanks and involves the use of mixers to bring substrate in constant contact with the microorganisms, as well as the optimization of all the environmental and loading-rate factors.

Small biogas tanks are normally not agitated completely, nor do they include all the components of a high-rate digester, such as heat exchangers, supernatant and sludge draw-off and vacuum relief-valves. Figure 3 shows three typical designs of small biogas plants that are operating in Asia. Small biogas tanks for individual households or small farms vary in design from area to area and country to country. They are mainly empirically sized and are constructed of local materials. Besides producing biogas energy, these tanks serve as a sanitary method of waste storage and thus improve public health sanitation standards around the farms.

DIGESTER START-UP. There is no need to add any special mixture of bacteria to initiate the process in an anaerobic digester. However, to speed up the process at the beginning, it is a common practice to seed the tank with actively digesting sludge from a sewage-treatment plant, or from a well-digested manure pit. For large digesters, it might be a good idea to mix the incoming fresh, raw waste with digested sludge for the first two weeks or so until uniform gas production is achieved. This type of seeding may also be used in restarting a digester that has gone "sour" (excessive accumulation of volatile acids; low pH, and/or low $CH_4$ to $CO_2$ ratio).

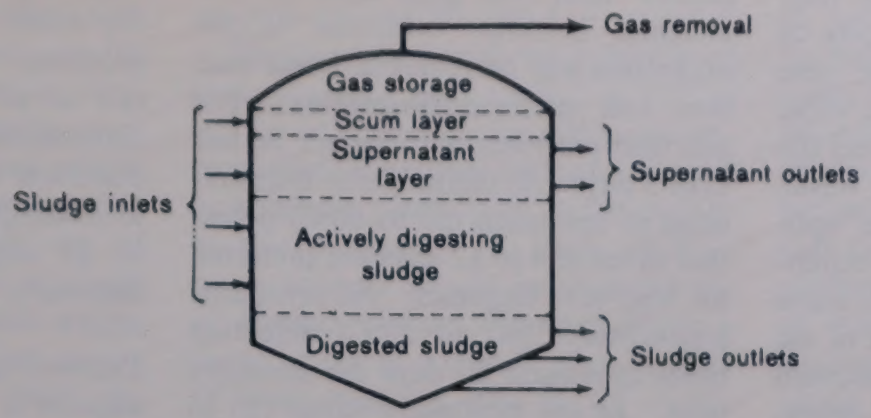
TEMPERATURE CONTROL. Temperature control is provided by heat exchangers that heat the sludge either outside the tank or by circulating hot water throughout the tank contents. Mean specific heat for sludge may be assumed to be equal to 1.0 for concentrations of solids of less than 10 percent. Temperature control in large digesters through heat exchangers requires good, reliable equipment and continuous maintenance. In warm climates, where there are no freezing temperatures, digesters may be operated without added heat, but the detention time needs to be increased. Gas production is definitely affected by temperature of operation.

Figure 4 shows typical relationships between gas production and temperature of operation at various detention times. At 12 days of detention time, gas production per unit of organic matter (TVS) added to the digester per day is 20 percent higher at 45°C than at 35°C (1.20 *vs* 1.0 relative gas volume). At an operating temperature of 25°C, Figure 4 indicates the gas volume produced would be 25 percent less than at 35°C. At 55°C gas production is 50 to 60 percent higher than at 35°C. Gas production decreases rapidly if temperatures go beyond 60°C, because at such high temperature the bacterial die-off rate is also high. At the low temperature of 15°C, gas production is half as much as at 35°C and one fourth as much as at 55°C. Even though gas temperature level is important, changes in digester temperature are more critical. Methane bacteria are sensitive to sudden temperature changes. A drop of only a few degrees in the temperature at which the methane bacteria culture has been working will frequently arrest the production of gas without materially affecting production of acids. This may result in dangerous accumulation of acids. Temperature must, therefore, be controlled within a narrow range (2 to 5°C). To protect the process against a possible malfunctioning of the temperature-control system and also to cut down on the energy inputs, it is good practice to insulate the tank as much as possible. One way to insulate the digester is to pile soil around it.

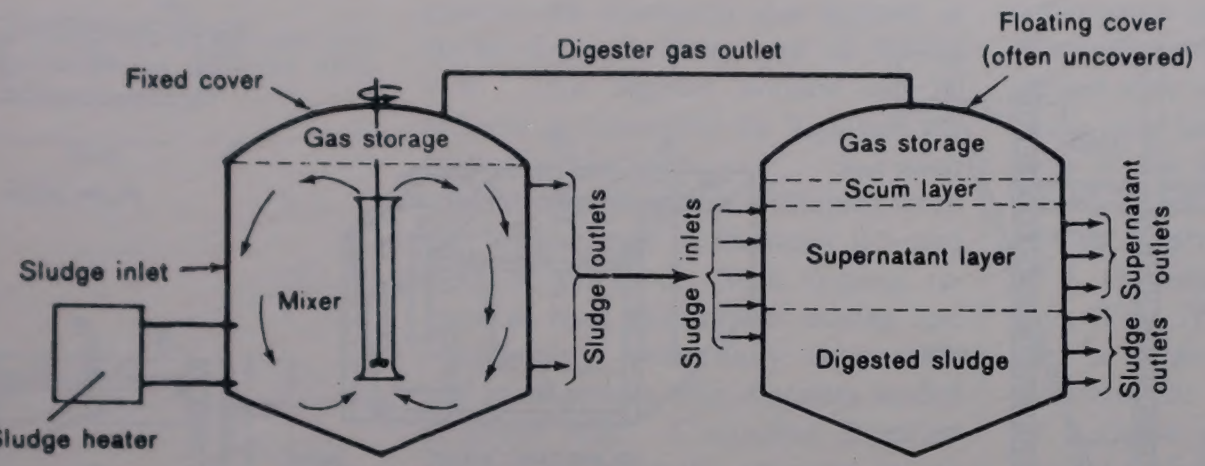
DETENTION TIME. Detention time is the number of days that the waste remains in the digester before it is drawn off as supernatant fluid or as digested sludge. In a completely mixed digester the hydraulic detention time (HDT) is equal to the volume of the digester divided by the volume of waste inflow. For example, if the waste flow is 10 $m^3/d$ and the digester has a liquid volume of 100 $m^3$, then HDT is 10 days. Because waste-waters contain solids that settle out of the liquid (Fig. 2a), detention times for the solids and for the liquids may be different. Solids retention time (SRT) and HDT are the same in a completely mixed digester, but would be different in an unmixed digester unless the draw-off of supernatant and digested sludge solids is such that the concentration of the solids of the removed waste is the same as that entering the digester. Normally, when the term "detention time" is used, it refers to HDT unless SRT is specified.

Detention time must be decided upon by the designer after experimentation and considering other parameters to which HDT is mathematically related. As Figure 4 illustrates, the volume of gas produced per unit of waste increases rapidly with increase in detention time (HDT) from 4 days to about 10 days in a high-rate digester, and tapers off with increases in HDT. Optimum detention time depends on the waste to be digested, temperature of operation, type of digestion (high-rate *vs* conventional), and on eco-

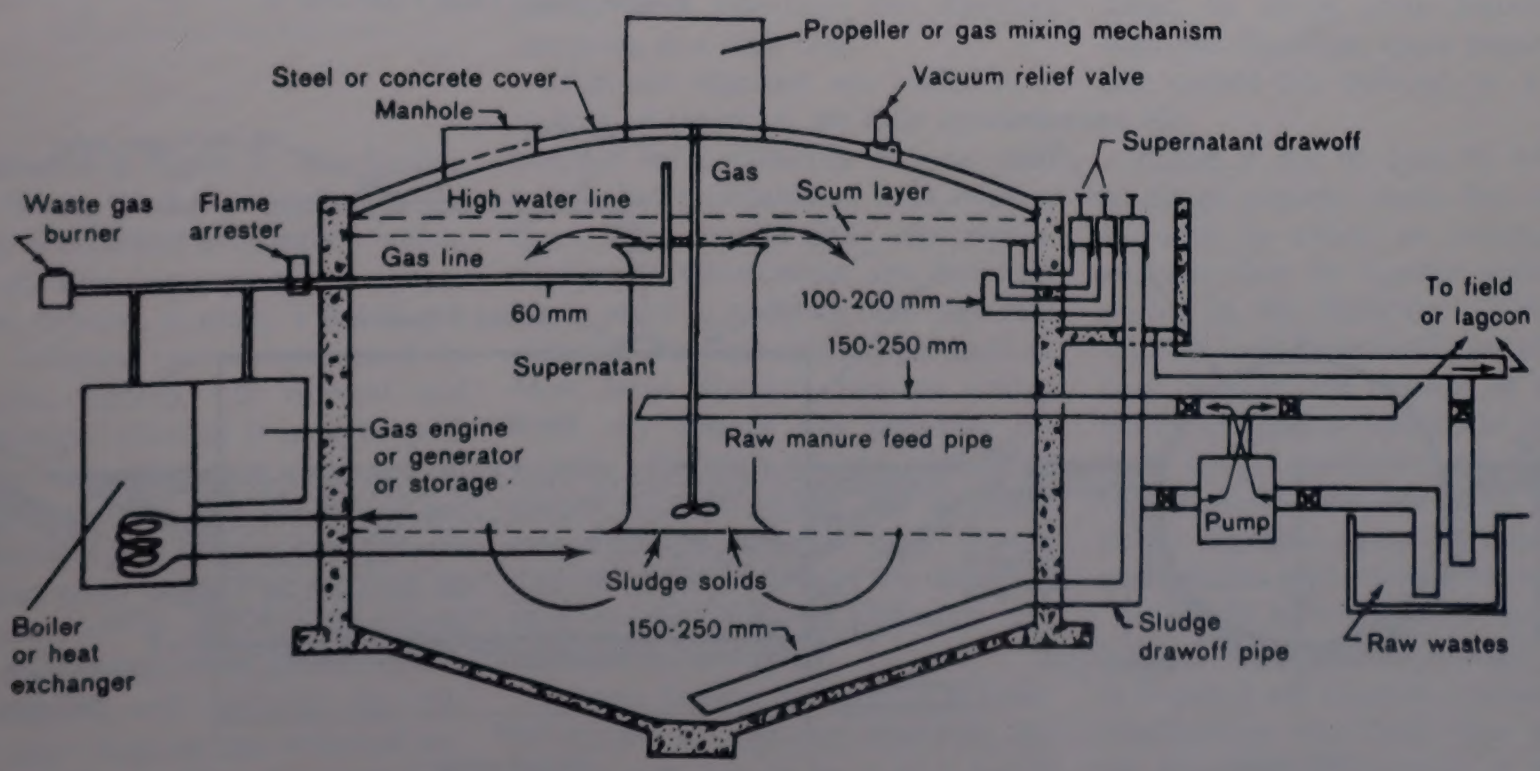
**Figure 2.** *Schematics of large digesters*




STANDARD, CONVENTIONAL DIGESTER




TWO-STAGE HIGH-RATE DIGESTER



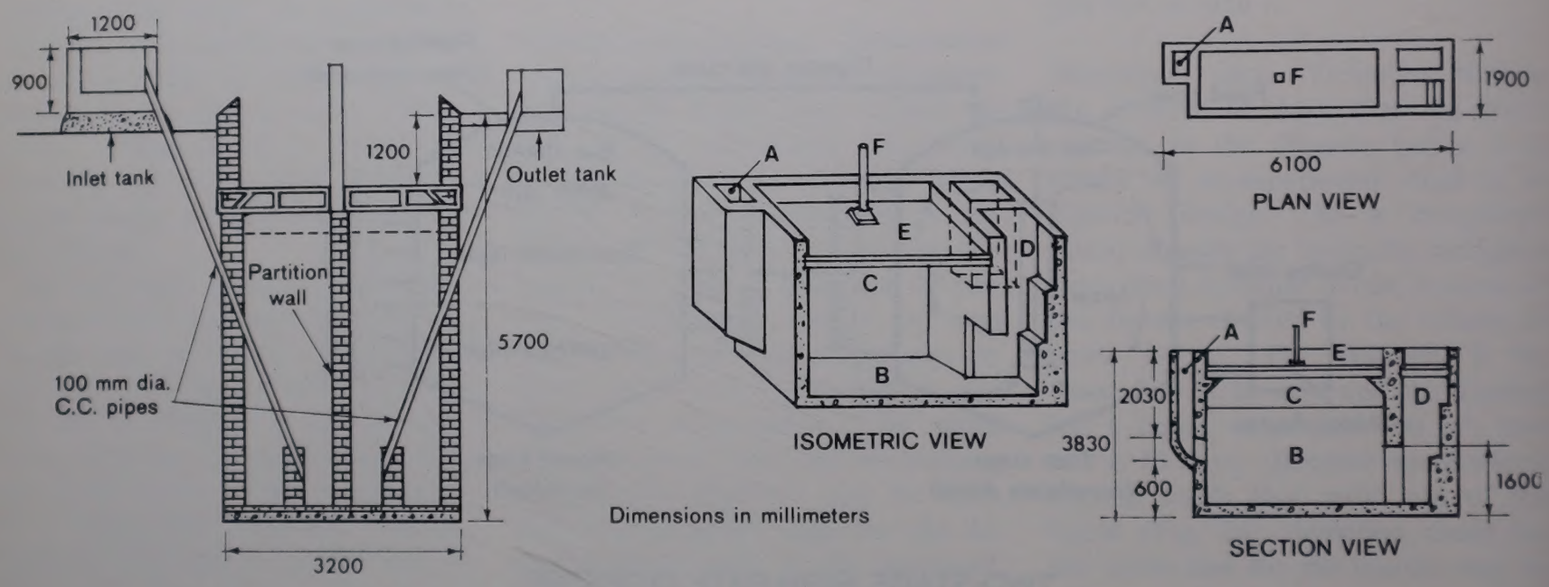

MAJOR COMPONENTS OF A COMPLETE DIGESTER

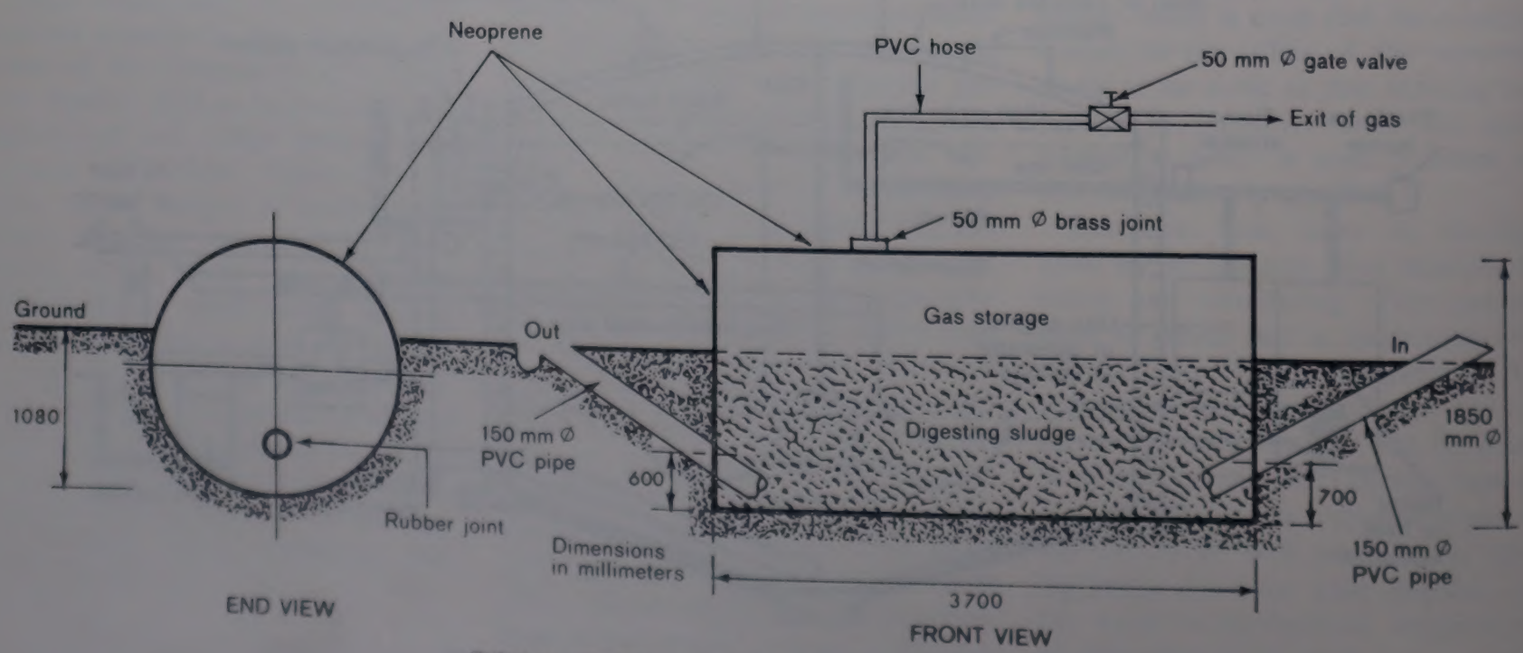
nomics. Longer detention time means bigger tanks, which cost more than small tanks. Therefore, the costs of constructing digesters of large size have to be balanced against the value of the additional gas produced and the value of added storage for the waste. However, if digestion is to be optimized, detention time must be experimentally determined using the waste itself. The solids concentration of the waste does affect HDT as is illustrated in Figure 5 and will be discussed below.

Minimum detention time must be greater than the generation of the methane bacteria, otherwise microorganisms will be removed faster than they can produce themselves. For practical purposes, HDT cannot be less than 5 days. At thermophilic temperatures of operation (50 to 60°C) detention times of 8 to 12 days are preferred for high-rate digesters. At cryophilic temperatures (10 to 20°C) detention times of around 30 days are normally used. In the mesophilic range (25 to 45°C) HDTs of 10 to 20 days are practical, depending on the type of waste. Pig waste, which is high in easily degradable, volatile solids, requires an HDT of around 10 to 12 days, while cattle waste, which is high in cellulosic materials and fibres that are difficult to decompose, requires an HDT closer to 20 days (Bousfield, Hobson and Summers, 1979).

LOADING RATE. The rate at which the digester is fed is critical. Mixing, and

**Figure 3.** *Three typical Asian designs of biogas plants*

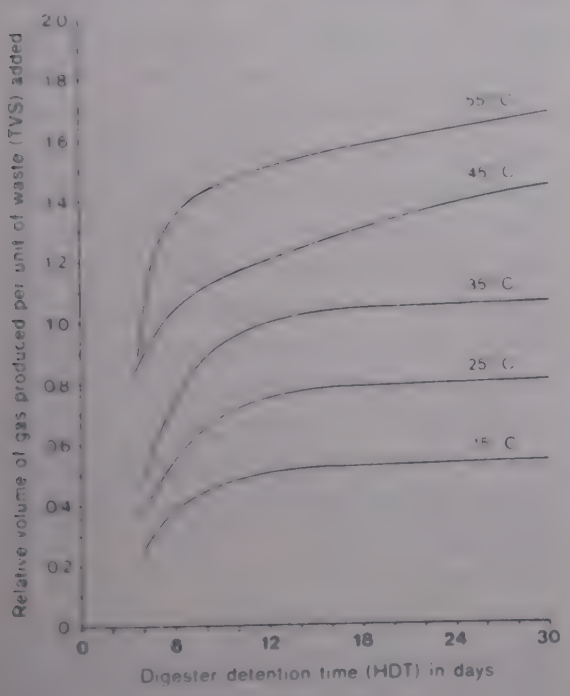


the volumetric loading rate (VLR) determine the type of digestion, i.e., conventional or high-rate. Theoretically, loading rate should be based on the concept of a food-to-bacteria ratio (F:M). In practice the loading rate is expressed in terms of either weight of volatile solids (TVS) added per day per unit volume of digester (kg of TVS/$m^3$-d of digester volume) or weight of TVS added per day per unit weight of TVS in the digester. For design purposes the first is used while the latter is useful in the monitoring of the operation of a digester.

For easy reference digestion is classified according to the loading rate as

**Figure 4.** *Gas production at different temperatures of operation and detention times*




is illustrated in Figure 5. For loading rates below 0.2 kg of TVS/$m^3$-d, the system is classified as a lagoon which, although this may operate as a continuous process, is really a batch system. Methane gas is generated in anaerobic lagoons. In tropical and semi-tropical climates it may be possible to collect the gas produced in anaerobic lagoons, but the rate of gas production is low. Also, it is difficult to collect gases from large surfaces, as Nordstedt *et al.* (1976) discovered when they constructed plastic covers over lagoons and analysed the collected gas. Lagoon gas collected averaged 60 percent $CH_4$, 7 percent $CO_2$ and 31 percent air. Gas production ranged from 6 to 400 l/d per square metre of lagoon surface. The average was around 40 l/$m^2$-d.

Digester tanks with no agitation of tank contents, and loaded at 0.2 to 0.5 kg of TVS/$m^3$-d constitute an uncontrolled digestion system. Small-farm biogas plants (Fig. 3) fall mainly in this category. In terms of how many animals should be used per $m^3$ of digester volume, Figure 5 shows a range of 0.4 to 1.0 pork pigs, or 6 to 15 layer hens, or 0.04 to 0.1 cattle. This relationship between loading rate and number of animals is based on the rate organic matter (TVS) is excreted per farm animal of 100-kg total liveweight (TLW). For uncontrolled digestion, the minimum size digester is 10 $m^3$ for each dairy cow of 500-kg TLW. This digester volume can be reduced if controlled or high-rate digestion is practised.

Controlled digestion is attained when the loading rate is between 0.5 and 2 kg of TVS/$m^2$-d. Such systems require at least intermittent mixing and considerably more daily supervision and management than digesters loaded at lower rates. Controlled digestion systems are normally operated in scientific institutes or in places where management skills have been developed. The pilot-plant-size digesters reported on in popular articles fall mainly in this category, but the potential of the process is extrapolated mainly from laboratory-size digesters operating at a high rate.

High-rate digesters are loaded at 2 to 6 kg of TVS/$m^3$-d, are fully agitated, and are fed continually at least once a day, but preferably at least twice a day. They require daily supervision, continuous maintenance and monitoring in order to maintain both high-rate digestion and high-rate gas production. Most large digesters treating sludge from city sewage are high-rate digesters. Statistics on gas production quoted in most papers are for high-rate digestion systems, even though data on the loading rate, the detention time or the mode of operation are included.

As may be seen in Figure 5, in order to operate at high-rate digestion the loading rate has to be 2 to 6 kg of TVS/$m^3$-d at an optimum detention time of 10 to 15 days, and the concentration of organic solids (percentage of TVS) in the incoming waste has to be between 2 and 9 percent on a wet basis. Since TVS constitute around 80 percent of the total solids (TTS), this would mean a TTS content of 2.5 percent to 11 percent [moisture content (TMC) of 98 percent down to 91 percent]. The content of total solids of animal manures as excreted (including urine and faeces) ranges from about 9 percent TTS (91 percent TMC) for dairy cattle to 30 percent TTS (70 percent TMC) for sheep. Thus, animal manures have to be diluted somewhat to bring their TTS below 15 percent (85 percent moisture) in order to accommodate good digestion (Fig. 5). Normally, even dairy cattle waste is diluted with water before digestion because only faecal solids are collected, most of the urine being lost. If the TVS of the effluent waste is 10 percent and the loading rate is 2 kg of TVS/$m^3$-d, then the detention time is 50 days (Fig. 5). Since 50 days is too long a detention time for high-rate digestion, the waste has to be diluted by a factor of five in order to get down to 10 days of HDT, and 2 kg of TVS/$m^3$-d.

In high-rate digestion, the digester volume required per animal is reduced. Wastes from 4 to 12 pigs, 60 to 180 hens, and 0.4 to 1.2 dairy cattle can be digested per $m^3$ of digester volume. Thus, by going from lagooning to high-rate digestion, space requirements per animal are reduced by a factor of 60.

Figure 5 can be used by engineers to design digester tanks, but can also be used by others to compare and evaluate data on digester systems reported in the literature. Using Figure 5, it is mathematically possible and easy to relate the number of animals per $m^3$ of digester capacity to the three main design parameters of digestion: volumetric loading rate (VLR); hydraulic (or digester) detention time (HDT); and volatile solids concentration (TVS).

It should be noted, before going any further, that the relationships shown in Figure 5 are physical, mathematical relationships that hold true whether the digesters are operated at 25°C or at 55°C. It is the gas yields and man-

agement requirements that increase as temperature level of operation increases. Figure 5 is useful in indicating that papers on digestion that do not give data on at least two of the three critical design parameters (VLR or number of animals per $m^3$, TVS or TTS, and HDT) and temperature of operation are not complete and may even be useless. If the volume of the tank and VLR are given in a published paper, then the HDT can be calculated by dividing the tank volume by VLR. From this, Figure 5 can be used to back-calculate the concentration of solids in the waste. Concentration of solids is important in the digestion process.

CONCENTRATION OF SOLIDS. Figure 5 shows that concentration of solids is related to other critical parameters, as has already been shown. It is desirable to note again, however, that concentration of solids of 3 to 10 percent is preferable for both high-rate and controlled digestion. Bousfield, Hobson and Summers (1979) found that gas production increased 40 percent when concentration of solids (TTS) of cattle waste increased from 5 percent to 10 percent. But the same researchers found that gas production decreased almost 60 percent when poultry waste was used and concentration of solids increased from 5 to 12 percent. At high concentrations of solids, particularly with poultry waste, high ammonia concentrations inhibit the digestion process.

The optimum concentration of solids in the digester depends on the type of the waste used. It is critical, therefore, before establishing the level of solids at which the digester will operate, to run experiments to find the level that gives the best gas yields; that is, if gas production is the main goal. As is possible to deduce from Figure 5, if the concentration of solids is too low, high loading-rates cannot be achieved within optimum detention times. Also, if the digester is to be heated, the heating requirements become unnecessarily high. However, if the concentration of solids is too high — say, over 15 percent TVS — then digestion has to be carried out in long HDT times. Furthermore, keeping all the solids in suspension could be a problem in high-rate digestion. Moreover, at very high concentrations of solids, volatile acids and $CO_2$ content of the gas tend to increase. It is preferable to operate the digester at a concentration of solids that is the highest possible without creating adverse effects on the production of volatile acids. In this case also the energy requirements for mixing the digester contents should be considered.

**Figure 5.** *Biogas digester capacities required for various animals at different levels of digester operation. [Assume 70% collection of TVS and mean liveweight (TLW) as 60 kg for pigs, 2 kg for hens and 500 kg for cattle.]*

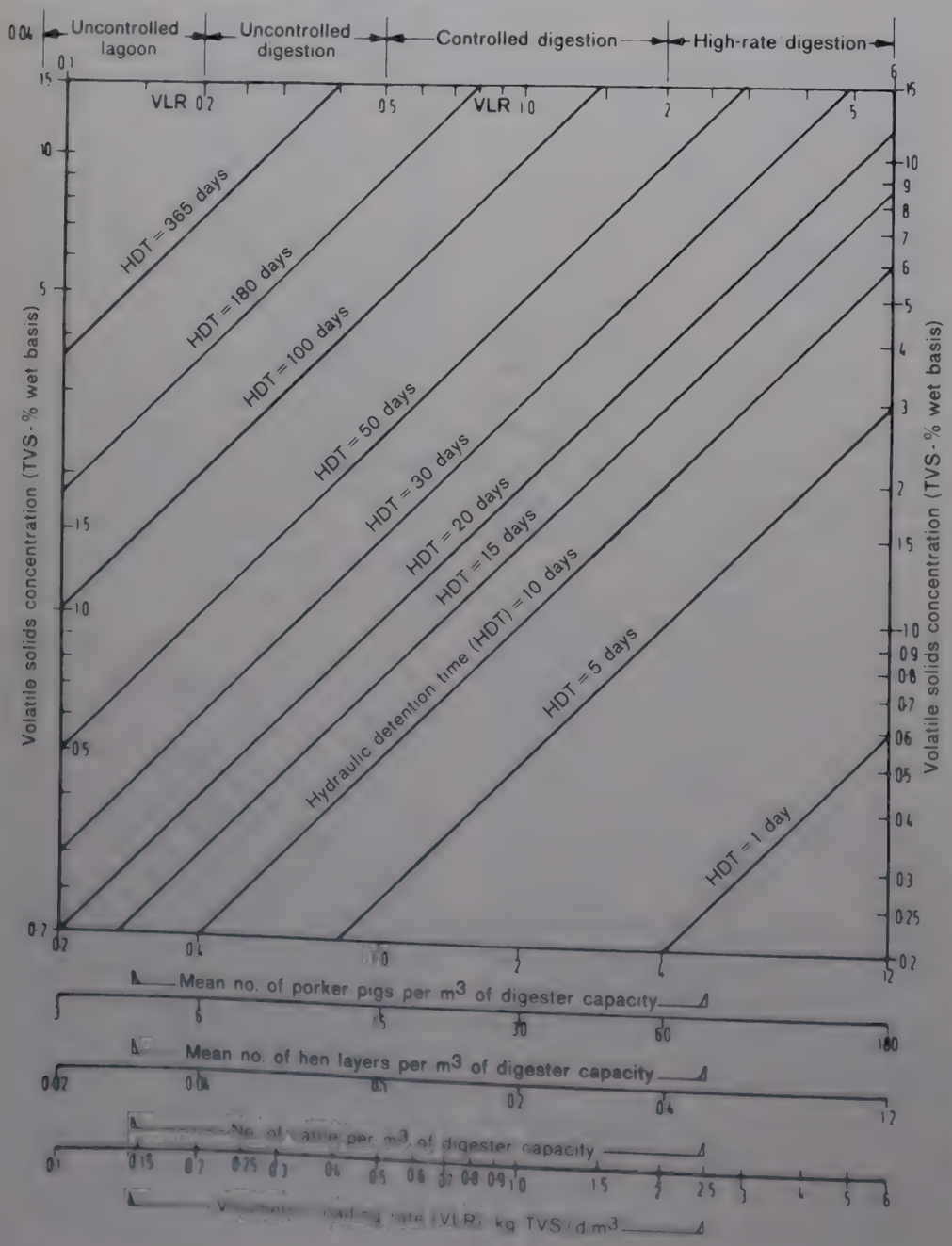

Uncontrolled lagoon
Uncontrolled digestion
Controlled digestion
High-rate digestion
VLR 0·2
VLR 1·0
HDT = 365 days
HDT = 180 days
HDT = 100 days
HDT = 50 days
HDT = 30 days
HDT = 20 days
HDT = 15 days
HDT = 10 days
HDT = 5 days
HDT = 1 day
Hydraulic detention time (HDT)
Volatile solids concentration (TVS - % wet basis)
Volatile solids concentration (TVS - % wet basis)
Mean no. of porker pigs per m3 of digester capacity
Mean no. of hen layers per m3 of digester capacity
No. of cattle per m3 of digester capacity
Volatile solids loading rate (VLR) kg TVS/d.m3

TABLE 1. **Animal waste properties**

| Waste property | Symbol | Unit | Pigs | Layer hens | Feedlot beef | Feedlot sheep | Dairy cattle |
|---|---|---|---|---|---|---|---|
| Excreted wet waste | TWW | kg/d of APU [1] | 5.1 | 6.6 | 4.6 | 3.6 | 9.4 |
| Urine | U | % of TWW | 45.0 | 0 | 29.0 | 50.0 | 31.0 |
| Total solids | TTS | % of TWW | 13.5 | 25.3 | 17.2 | 29.7 | 9.3 |
| | | kg/d of APU | 0.69 | 1.67 | 0.70 | 1.07 | 0.89 |
| Moisture content | TMC | % of TWW | 86.5 | 74.7 | 82.8 | 70.3 | 90.7 |
| Total volatile solids | TVS | % of TTS | 82.4 | 72.8 | 82.8 | 84.7 | 80.3 |
| | | kg/d of APU | 0.57 | 1.22 | 0.65 | 0.91 | 0.72 |
| Total Kjeldahl nitrogen | TKN | % of TTS | 5.6 | 5.9 | 7.8 | 4.0 | 4.0 |
| | | kg/d of APU | 0.039 | 0.099 | 0.055 | 0.043 | 0.036 |

[1] APU = Animal Population Unit, which is equal to 100 kg of total liveweight (TLW) of the animal, i.e., 50 chickens each of 2 kg of TLW would make one APU.

of TVS and high energy content in the gas (70 percent of $CH_4$).

WASTE PROPERTIES. Animals excrete waste in proportion to their liveweight. Table 1 gives suggested guideline-values for waste properties that are relevant to biogas plant design (Taiganides, 1977). Values and detailed explanations of other parameters, such as contaminating metals and pollution potential of animal wastes, are given in Taiganides (1977). The most important design property is the organic matter content (TVS) of the waste and its concentration in the diluted waste. TVS values are, of course, related to the total wet waste, TTS, TMC, etc., as has already been shown and as can be seen in Table 1. Table 1 gives the amount of TVS excreted per animal population unit (APU) per day, APU being defined as an animal with a total liveweight (TLW) of 100 kg. The term APU makes it possible to determine the equivalent of wastes from animals of different size (since it is meaningless to refer to "pigs" or even "average size pig" without specifying their average TLW). The term makes it easy to calculate waste-property values and compare them with values for other animal species.

From Table 1 and Figures 4 and 5, an engineer can make a preliminary design of a digester. Using such preliminary designs, it is then possible to determine the basic economic feasibility of the project and of various alternatives in terms of levels of temperature, detention time, dilution or concentration of the solids in the waste, etc. After these preliminary designs, it is important that, for large projects, actual tests be made to optimize the parameters, as has already been outlined, taking into consideration the availability of materials, equipment and management skills.

## Methods of monitoring digester operation

Once a digester is built, the question is, how does one determine whether it is operating well or whether it is a success or not? Of course, any estimate of the success of a system depends on what the objectives of the project were and the priority placed on each of the objectives. As has already been indicated, the objectives may be many: gas energy recovery, stabilization of solids, sanitation/pathogen control, development of skills in bacteria husbandry, etc. Assuming that energy recovery is of the highest priority, then the parameters to monitor are: volatile acids, pH, bicarbonate alkalinity, gas production and gas quality.

Since pH is easy to measure, it should be monitored routinely. A decline of pH below 6.5 is as good an indicator as any that the digestion process is in trouble. If the pH rises above 7.6, then ammonia concentration should be checked. Ammonia can reach levels that can inhibit methane production. If the digester is operated at a research institute equipped with instrumentation for chemical analyses, then routine tests of bicarbonate alkalinity and volatile acids should be made. Volatile acids should stay below 500 mg/l as acetic acid for a digester functioning properly. If the volatile acids exceed 2 000 mg/l, then methane production will be seriously inhibited. Bicarbonate alkalinity should be in the range of 1 500 to 5 000 mg/l as calcium carbonate ($CaCO_3$). Standard procedures for these tests and for all other waste properties such as TVS, TTS and TKN (Table 1) are given in APHA (1975). Whenever possible, only standard test procedures should be used. Another way to check the stability of the process without using equipment is to monitor the quality of the digested sludge. Unstabilized sludge smells worse than digested sludge and will not be as black. An experienced operator can easily differentiate between the two.

GAS PRODUCTION. Gas production is the best indicator of an impending malfunction of the process. In animal-waste digestion, gas production should be averaging around 0.55 $m^3$/kg of TVS added to the digester when the digester is operating at around 35°C. Maximum gas production at that temperature could reach 1 $m^3$/kg of TVS added for a high-rate digester. At lower temperatures, say around 25°C, maximum gas production would not be more than 75 percent of that at 35°C and less than 50 percent at 15°C (Fig. 4). Another common way of expressing gas production is in terms of $m^3$ of gas per kg of TVS destroyed. In a well-operating digester about 50 percent of the TVS input would be destroyed. Therefore, gas production should average around 1 $m^3$/kg of TVS destroyed. The maximum should be around 2 $m^3$/kg of TVS destroyed. These are general figures that should be used as guidelines of what to expect when monitoring a digester, or when evaluating results reported in the literature.

If the amount of TVS added to the digester per day cannot be accurately estimated, then gas production may be measured in $m^3$ of gas produced per day per $m^3$ digester volume, which is a ratio of volumes, i.e., gas volume to digester volume. This ratio would be around 1:1, with a range of up to 4:1 in high-rate digesters, and as high as 10:1 in small experimental digesters operating at the thermophilic range.

GAS QUALITY. If measuring equipment

TABLE 2. **Heat of combustion of biogas and other competitive fuels**

| Energy material | Calories equivalent (kcal) | Electricity equivalent (kWh) | 1 m³ biogas equivalent |
|---|---|---|---|
| *Solid fuels* (per kg) | | | |
| Lignite | 7 270 | 8.46 | 0.72 |
| Anthracite | 7 050 | 8.20 | 0.74 |
| Coal coke | 7 220 | 8.39 | 0.72 |
| Briquets | 6 380 | 7.42 | 0.82 |
| Bagasse (12% $H_2O$) | 4 040 | 4.70 | 1.29 |
| Bamboo (10.5% $H_2O$) | 4 110 | 4.78 | 1.27 |
| Wool (12% $H_2O$) | 4 300 | 5.00 | 1.21 |
| *Liquid fuels* (per l) | | | |
| Crude oil | 9 680 | 11.26 | 0.54 |
| Kerosene | 8 992 | 10.46 | 0.58 |
| Gasoline | 8 400 | 9.88 | 0.61 |
| *Gaseous fuels* (per m³) | | | |
| Natural gas | 9 340 | 10.87 | 0.56 |
| Manufactured gas | 4 900 | 5.69 | 1.06 |
| LPG - Liquefied Petroleum Gas | $6.6 \times 10^6$ | 7 701 | $0.8 \times 10^{-3}$ |
| *Biogas* (per m³) 65% $CH_4$, 35% $CO_2$ | 5 200 | 6.00 | 1.0 |
| Purified biogas (100% $CH_4$) | 8 010 | 9.31 | 0.65 |
| *Electrical energy* (per kWh) | 860 | — | 6.0 |

is available (APHA, 1975) the $CO_2$ content of the gas (or the $CH_4$:$CO_2$ ratio) may be used as another indicator of the stability of the process. When $CO_2$ content begins to increase beyond 40 percent of the gas, the process may be running into trouble.

In a well-operated biogas plant, the gas composition is 60 to 75 percent $CH_4$, 40 to 25 percent $CO_2$ plus trace quantities of $H_2S$, $N_2$, $NH_3$ and volatile gases. The energy is mainly from the methane ($CH_4$) of the gas, while the distinct odour of rotten egg is from the trace quantities present of hydrogen sulphide ($H_2S$). The heating values of biogas and of other materials used as energy sources are given in Table 2. Removal of $CO_2$ from the biogas would raise its heating value to that of methane (around 1 000 kcal/m³). Biogas produced by a digester that is functioning well has around 65 percent $CH_4$ on average and will have a heating value of about 5 200 kcal/m³. As is shown in Table 2, this is equivalent to about 6 kWh in terms of electrical energy. Table 2 shows the heat content of most of the common fuels used as energy sources in rural areas. The last column of Table 2 gives the heat energy of other fuels equivalent to 1 m³ of biogas of 65 percent $CH_4$. It should be noted that biogas is competitive with all sources of energy, particularly manufactured gas. Economic competitiveness of the biogas will depend on the local prices of the other fuels, the cost of biogas, and the availability and reliability of each energy source. Denuding forests to provide energy for cooking is not acceptable no matter what the short-term costs may be. In other words, the price of other sources of energy may be lower than biogas but their availability cannot be ensured; biogas is thus competitive, even though it may cost more.

TABLE 3. **Approximate quantities and heat value of biogas from waste**

| Waste | TLW (kg) | Gas production (l/kg of TVS) | Gas production (l/day/animal) | Methane (%) | Heat value (kcal/day) |
|---|---|---|---|---|---|
| Human | 50 | 450 | 37 | 70 | 225 |
| Laying hen | 2 | 450 | 11 | 70 | 62 |
| Fattening pig | 50 | 630 | 180 | 65 | 900 |
| Dairy cow | 500 | 330 | 1 200 | 70 | 6 850 |

GAS YIELD. The usual question asked is how much gas can be expected from the anaerobic digestion of animal wastes. As has been repeatedly emphasized, gas yield depends on many factors, prominent among which are temperature, rate of digestion and type of waste. However, Table 3 sets out gas yields as guideline-values and for purposes of comparison when monitoring a digester operation, or for referring to published literature. The values reported in Table 3 are for net gas volume and net heat values after subtracting an average of 30 percent of the gas produced for the maintenance of mesophilic temperature (35°C) in the digester (Taiganides *et al.*, 1963). The values given in Table 3 are higher than would be expected from uncontrolled digestion, because they are based on high-rate digestion. ■

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# Increasing animal production from sorghum forage

**J.L. Wheeler**

Sorghum species are cultivated for grain to a varying extent in almost all tropical and subtropical countries and their use for forage is almost as widespread. In 1974 over 42 million hectares were sown for grain (FAO, 1975) but data on the area used for forage are not available. Forage sorghums are $C_4$[1] plants and are notable for their high yield of dry matter, their capacity for regrowth and their efficient use of water. As $C_4$ plants they are presumably also more efficient in terms of dry matter produced per unit of nitrogen (N) than are $C_3$ grasses on soils of low N-content (Brown, 1978). Most forage sorghum is grazed, or cut and carried to animals. In some countries, particularly in the United States, it is also used for silage or hay and some is allowed to stand over for use in winter (Burns and Wedin, 1964; Rieck, Croy and Davies, 1972). In dry areas a little may be cut and left in the field (Cory, 1963).

In terms of animal performance, however, even where crop growth is satisfactory, the experience of farmers and extension workers is that, although carrying capacity may be high, production per head by unsupplemented animals on forage sorghum is often mediocre or poor. Indifferent animal performance is more frequently recognized by farmers in Australia because the feeding of supplements (grain/protein and minerals) to grazing animals is uncommon and hence does not mask the deficiencies in the forage, but it is also observed in Africa and North and South America (Hoveland, Anthony and Scarsbrook, 1967; J.C. Rodriguez Rey — personal communication). The purpose of this article is to discuss factors that contribute to unsatisfactory animal production from this agronomically desirable crop and to suggest management and research strategies to overcome them. The factors are the hydrogen cyanide potential, the sulphur and sodium contents and the digestibility of the forage. It is assumed that, except for milk production, nitrogen content is not a limiting factor although it may well be so if the forage has been allowed to become mature and opportunities for selective grazing are limited. Most of the published information concerns the sorghum × sudangrass hybrids (*Sorghum bicolor* × *S. sudanense*) but reference will also be made to sudangrass (*S. sudanense*) and Columbus grass (*S. almum*).

*Dairy cows grazing sorghum × sudangrass hybrid near Brisbane, Queensland*

**Hydrogen cyanide potential.** All sorghums contain at least trace amounts of a cyanogenic glucoside. After the plant is ingested, hydrogen cyanide (HCN) is released by the action of an enzyme (β-glucosidase). The enzyme is usually present in plant tissue and is, in any event, present in rumen liquor (Coop and Blakley, 1949). As free HCN is not present in the plant it is customary to refer to the HCN potential (HCN-p) of the plant. Kingsbury (1964), discussing the HCN-p of sorghum species, quotes a maximum of 0.34 percent (DM basis) but commercial crops are commonly in the range of 0.02-0.08 percent. Discussions of cyanogenic glucosides and of their possible role in the plant are given by Conn (1969), Jones (1972) and Tapper and Reay (1973). It has been suggested (see Jones, 1972) that the capacity to release HCN when plant cells are ruptured is a defence mechanism against insect and possibly fungal attack. There is no direct evidence that this is the case with

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[1] $C_4$ plants use a pathway for photosynthesis, characteristic of tropical grasses, that involves four-carbon acids. In hot, bright conditions it is much more efficient than the three-carbon pathway ($C_3$) used by temperate grasses.

sorghums. However, Tift sudangrass, selected for disease resistance (Burton, 1943) is notably higher in HCN-p than other sudangrasses (Gangstad, 1959; Gorz *et al.*, 1977) and modern sorghum × sudangrass hybrids bred largely for yield and fungal-disease resistance continue to have high HCN potentials. In studies of the genetics of cyanogenesis in sorghum (Carlson, 1958; Nass, 1972) some association between disease resistance and HCN potential was noted.

In addition to genotype the HCN-p of sorghums is substantially affected by the environment of the plant and its stage of growth. Factors that unequivocally increase the HCN-p of a given plant are high available nitrogen (Pinckney, 1924); inadequate phosphorus supply (Wheeler *et al.*, 1979); shortage of moisture (Willaman and West, 1916; Heinrichs and Anderson, 1947); injury to the plant (Franzke and Hume, 1945) and any management practice that results in a high proportion of leaf in relation to stem (Wolf and Washko, 1967). Light frosts appear to increase HCN-p and killing frosts to decrease it but the literature is not conclusive (Wattenbarger *et al.*, 1968). Future work needs to distinguish between the effect of frost on the content of glucoside and on the content and activity of the hydrolysing enzyme. Herbicides probably do not affect HCN potential (Brown and Beaty, 1970). A long-held but not well-established belief is that aphid attack increases the HCN-p in sorghum (Balfour, 1903). Rose (1941) observed that occurrences of fatal HCN-poisoning were almost always associated with attacks on the sorghum by grasshoppers (locusts). However, several factors other than partial defoliation would have been involved and no experimental study has been reported.

The presence of cyanogenic glucosides in sorghum may adversely affect animal production in four ways.

*HCN poisoning.* Poisoning of all classes of livestock has long been known: "numerous instances" were reported in Australia in the 1890s (Anon., 1897). However, the number of deaths is very low in relation to the number of animals that safely graze sorghum each year, particularly with the therapeutic measures in use for the last four decades (Chen and Rose, 1952). Intravenous injection of sodium nitrite and sodium thiosulphate followed by oral doses of sodium thiosulphate is an effective treatment (Blood, Henderson and Radostits, 1979).

*Fear of poisoning.* The fear that farmers have of their animals being poisoned undoubtedly causes greater monetary loss than the actual deaths as it leads to stock being withheld from grazing while the forage is leafy and nutritious and to stock not being allowed to graze the stubbles of grain-sorghum fields (Macadam, 1970).

*Reduced intake.* There is some evidence that sorghums with high HCN-p are less acceptable to sheep and cattle than those with lower levels (Rabas, Schmid and Marten, 1970; Hedges *et al.*, 1978). Low intake of forages under test conditions is not necessarily reflected in intake over longer periods by animals that are given no choice. Nonetheless, it is possible that high HCN-p, by reducing voluntary feed consumption, may contribute to poor production by animals consuming this forage. Hoveland (1969) notes two reports of low intake of sorghum-sudangrasses by stock, and Fribourg (1973) lists increased palatability and intake as necessary objectives in breeding forage sorghums. Said, Wheeler and Lindstad (1977) report a significant negative correlation between weekly weight gain by sheep and the HCN-p of their diet.

It seems probable that HCN in sorghum can bring about a reduction in forage intake as a consequence of the detoxication mechanism within the animal. The primary route for detoxication is the formation of thiocyanate, mainly in the liver through the action of the enzyme rhodanese using sulphide, thiosulphate or cystine as the sulphur donor (Blakley and Coop, 1949). It is not entirely clear which compounds normally act as sulphur donors (Williams, 1959; Oke, 1973). Further studies of this in ruminants ingesting cyanogenic forage and of the proportion of HCN detoxicated as

TABLE 1. **Sulphur and sodium content of forage sorghum (dry matter basis)**
*Percentage*

| Type of sorghum | Region grown | Sulphur | Sodium | Reference |
|---|---|---|---|---|
| Sorghum-sudan hybrids | Texas, USA | 0.10±0.12 | n.a. | Owen & Furr (1967) |
| Sudangrass hay | USA | 0.06 | 0.02 | NRC (1971b) |
| Sorghum (cv. Atlas and Sorgho) | USA | n.a. | [1] 0.02-0.11 | NRC (1971b) |
| Sorghum and sudangrass | NSW, Australia | 0.22-0.28 | n.a. | S.T. Dawe (pers. comm., 1976) |
| *Sorghum almum* | Kenya | 0.13-0.23 | 0.009-0.012 | Said, Wheeler & Linstad (1977) |
| Sorghum × sudangrass | Qld, Australia | 0.12-0.15 | 0.03 | Stobbs & Wheeler (1977) |
| Sorghum and sorghum × sudan [2] | NSW, Australia | 0.145±0.005 | 0.011±0.004 | Hedges *et al.* (1978) |
| Sorghum × sudangrass | NSW, Australia | 0.08-0.11 | 0.008-0.014 | Archer & Wheeler (1978) |
| | | 0.07-0.14 | 0.005-0.008 | Archer & Wheeler (1978) |
| Sorghum × sudangrass | New Zealand | n.a. | 0.01 | Smith, Middleton & Edmonds (1978) |
| Sorghum × sudangrass | New Zealand | 0.08-0.19 | 0.01-0.02 | Hunt, Taylor & Ness (1979) |

NOTE: n.a. = not analysed.
[1] Plus a questionable value of 0.36 percent of Na in fresh 'Sorgho' **cf.** 0.03 percent in 'Sorgho' silage. —
[2] Mean and SE of eight cultivars over four samplings. For comparison, in the same experiment pearl millet averaged 0.19 ± 0.009 percent of S and 0.024 ± 0.002 percent of Na.

TABLE 2. **Response to sulphur supplements by animals grazing sorghum forage**

| Measure | Type of sorghum | Method of administration | Production by control animals | Mean % response to sulphur | Significance | Reference |
|---|---|---|---|---|---|---|
| *Liveweight gain/head* | | | | | | |
| Sheep | Sorghum × sudan cv. sx11a | Lick | 96 g/d | 15 | n.s. | Wheeler, Hedges & Till (1975) Exp 1 |
| | | Lick | 107 g/d | 32 | * | Exp 2 |
| | | Lick | 45 g/d | 58 | ** | Exp 3 |
| | *S. almum* | 6 drenches/ week | 80 g/d | 1-22 | n.s. | Said, Wheeler & Lindstad (1977) |
| Beef cattle | Sorghum × sudan | Lick | 697 g/d | 14 | n.s. | Archer & Wheeler (1978) |
| *Milk production/cow* | | | | | | |
| Jersey cows | Sorghum × sudan | Daily drench | 7.5 kg/d | 6 | * | Stobbs & Wheeler (1977) |

NOTE: n.s. = Non-significant; * = significant $P < 0.05$; ** = significant $P < 0.01$.

thiocyanate are required. Quite large quantities of thiocyanate may be formed each day in animals consuming a full diet of cyanogenic forage, e.g., $>$ 1 g/d in sheep grazing white clover (Coop, 1949). Detoxication of HCN to $SCN^-$ requires theoretically 1.2 g of S/g of HCN. The quantities of sulphur sequestered for this purpose are probably nutritionally insignificant where the diet is high or adequate in sulphur, but, where the content of this element is marginal, the loss of sulphur can induce a deficiency.

Sulphur deficiency in animals causes, among other things, a loss of appetite (Whanger, 1972; Elam, 1975).

*Reduced production.* A reduction in intake will frequently reduce liveweight gain or milk production but there is a further consequence of cyanogenesis to be considered. The availability of methionine in the body is known to be at times the limiting factor in wool and other forms of animal production (Wright, 1969; Reis, 1979). Cystine exerts a sparing effect on methionine requirement and it seems probable that the loss of cystine for the detoxication of HCN will sometimes decrease tissue synthesis in the animal.

It is suggested, therefore, that the significant effects of cyanogenesis in sorghum lie not in the well-known poisoning, as this only rarely occurs in practice, but in its more insidious consequences. These are: a worldwide reluctance of farmers to use the forage while it is young, a depression in acceptability, a depression in voluntary feed intake and a reduction of the quantity of cystine or, more generally, of sulphur available for metabolism within the animal. The latter is of particular significance because, as will be discussed in the following section, the sulphur content of sorghum forage is often barely adequate or frankly inadequate for the animal's requirements.

**Sulphur.** Surprisingly few data on the sulphur content of sorghum forages have been reported. Table 1 gives data from a number of sources.

The sulphur requirements of ruminants are not very clearly defined (Whanger, 1972; Elam, 1975). In terms of the sulphur content of the diet, Bouchard and Conrad (1973) suggest 0.12-0.18 percent of DM for dairy cows, the NRC (1976) recommends 0.1 percent for cattle and Elam's (1975) review indicates a range from 0.1 to 0.17 percent for sheep. Sheep may respond more to sulphur than do cattle owing to differences in the efficiency with which this element is cycled (Kennedy, Williams and Siebert, 1975). The sulphur is required for synthesis of rumen microbial protein, which has a constant nitrogen:sulphur ratio of about 15:1 and Moir, Somers and Bray (1967) consider that the sulphur requirement of ruminants is better expressed as an N:S ratio. The optimal ratio cannot be determined precisely but as a guide they present evidence to show that, at least for sheep, the N:S ratio should be no greater than 10:1. Subsequently, Bird (1974) has suggested that for cattle diets a ratio of about 15:1 is adequate. Changes in the concept of the nitrogen requirements for ruminants arising from knowledge of protein degradation in the rumen (Kempton, Nolan and Leng, 1977) suggest that sulphur requirements may also have to be considered more in terms of the sulphur amino-acids available for absorption post-ruminally than in terms of dietary sulphur *per se*.

It is clear therefore that sheep and cattle on diets that consist solely of forage sorghum may be receiving inadequate levels of sulphur. The deficiency will be exacerbated in the case of forages with appreciable HCN potential. For example, in a study of the effect of fertilizers on, *inter alia*, the sulphur content of a sorghum × sudangrass hybrid the mean value for the second cut at a typical site was 0.159 percent of S (dry matter basis) and 2.71 percent of N (Wheeler *et al.*, 1980). The corresponding HCN-p was 0.072 percent (DM basis). Assuming this was all detoxified as thiocyanate the sulphur available for productive purposes in the diet would have been reduced to 0.071 percent and the effective N:S ratio would have widened to 38:1. In practice not all the HCN ingested is excreted as thiocyanate but clearly the ratio is greatly widened. Sulphur deficiency is associated with low average daily gain or milk production by stock (Elam, 1975). The extent to which poor animal production is a consequence of low forage intake or is a direct effect of actual or induced cystine deficiency at the tissue level is not clear.

A realization that there was an additional demand for sulphur created in ruminants when grazing cyanogenic sorghum forage led to a series of experiments on the effects of supplementation with sulphur. The results are summarized in Table 2.

It must be noted that the responses are variable although never negative. In some cases this is partly because of the variable intake of supplementary

*Young sorghum regrowth with a high cyanide potential*

S arising from the method of administering the supplement, but other factors are also responsible. For a positive response to occur there needs to be a wide N:S ratio in the diet brought about by the availability of non-protein nitrogen or by an intrinsically low sulphur content in the diet, or by a moderate sulphur content associated with a high HCN potential. Moreover, even if these conditions occur and dry matter intake is increased it is necessary that the additional forage ingested be of sufficiently high digestibility to promote weight gain or milk production. Sulphur fertilizers do not materially increase the sulphur content of sorghums (Owen and Furr, 1967; Hedges *et al.*, 1978; Wheeler *et al.*, 1980).

A further benefit that arises from ensuring that animals receive adequate sulphur in the diet is that it may reduce the risk of HCN poisoning. Although Coop and Blakley (1950), working with sheep, considered that, in normal circumstances, the sulphur donors do not limit the rate of detoxication, Oke (1969), reviewing the work of Himwich and Saunders (1948) and others, concluded that it is the rate of supply of sulphur that limits detoxication in the animal. Anderson (1956) gave 0.4 g of HCN/d to sheep on a diet containing 0.19 percent sulphur but could not give more than 0.1 g of HCN/d without causing distress to sheep on a diet containing 0.11 percent sulphur. It seems possible therefore that it is the low sulphur content of sorghum that gives rise to the world-wide reputation for the toxicity of the genus compared, for example, with the cyanogenic but non-toxic white clover (*Trifolium repens*) (Coop, 1949). In 1929 Steyn postulated that sulphur supplements would protect sheep from poisoning by bietou (*Dimorphotheca spectabilis*), a weed with high HCN-p and some protection was demonstrated. A subsequent experiment was not very conclusive and, unfortunately, the sulphur content of the diet was not recorded but sulphur-supplemented sheep produced more wool than the controls (Steyn, 1932). The author attempted to test the prophylactic effect of drenching sheep with sulphur but was unsuccessful: there were no signs of toxicity in 30 sheep drenched with 3 g of S/d (elemental or as sulphate) or in 120 undrenched sheep held for five days on wilted sorghum, 25-35-cm high with an HCN-p of 0.1 percent (DM basis). The sheep grazed the sorghum reluctantly, even after a week, without alternative feed.

The sulphur content of sorghum forage appears to be marginal or deficient for maximum animal production in many areas but further work is required. Liveweight gain and milk production have been increased significantly in some cases by supplementing the diet with sulphur. This may be done through the use of mineral licks or, in the case of cattle, in the drinking water.

**Sodium.** A study in East Africa on the effect on sheep grazing *Sorghum almum* of daily doses of sodium sulphate showed that, although under the conditions of the experiment there was no response to sulphur, the administration of 2.3 g of sodium (Na) and 1.9 g of potassium (K) per day increased liveweight gain over an eight-week period from 49 to 80 g/d (63 percent) (Said, Wheeler and Lindstad, 1977). The forage contained on average 0.01 percent of Na and 1.45 percent of K and, as the content of po-

*Cattle grazing forage sorghum, northern New South Wales* (PHOTO: D.J.B. WHEELER)

*A short hybrid sorghum in flower*

tassium was many times higher than required by sheep, the response was assumed to be to sodium. Response to sodium by steers lot-fed on sorghum grain had been reported earlier by Morris and Gartner (1971) but there were no records of cattle or sheep benefiting from sodium supplements while grazing sorghum. Unequivocal responses to sodium by grazing animals are not common and are generally restricted to animals grazing certain warm-season perennial grasses such as *Setaria anceps* cv. Nandi (T.R. Evans - personal communication), and those in native pastures in southeastern Queensland (Murphy and Plasto, 1973) and Papua New Guinea (Leche, 1977). A notable exception is the extensive series of experiments on lucerne containing 0.03 percent of Na grown on soils derived from pumice in New Zealand (Joyce and Brunswick, 1975): lambs supplemented with sodium gained carcass weight 98 percent faster than control animals and significant responses were also obtained with beef and dairy cattle.

Two series of experiments on the effect of sodium supplements on animals grazing sorghum have been conducted. In a two-year experiment the mean liveweight gain of cattle grazing a sorghum × sudangrass hybrid was increased from 571 g/d to 697 g/d by provision of a salt lick and to 781 g/d by a salt-plus-sulphur lick ($LSD_{.05}$ = 107 g/d) (Archer and Wheeler, 1978). The forage contained about 0.01 percent of Na and 0.12 percent of S. The wool growth of sheep grazing a sorghum hybrid (0.01 percent of Na) was increased from 9.8 to 10.9 g/d by dosing daily with 1 g of sodium ($P < 0.05$) but liveweight gain was not significantly increased (Wheeler and Hedges - unpublished data).

The sodium requirements of domestic animals have been comprehensively reviewed by Aitken (1976). Mechanisms for conserving sodium are very efficient. Horrocks (1964), for example, found that mature steers could tolerate a period of four months with intakes of $< 1$ g of Na/d. Lactation, however, represents a major drain of the element and Leche (1977) in Papua New Guinea has reported highly significant responses to sodium supplementation by lactating cows and their calves grazing pasture containing only a trace of sodium. The loss of sodium

(Left) *Sudangrasses at a highly digestible stage of growth, cv. Trudan on left, cv. Piper on right*

(Above) *A field of harvested sorghum in Somalia being grazed by camels belonging to members of the Galgail tribe*

(Right) *Sorghum growing with black gram* (Phaseolus mungo), *Manipur, western Nepal*

is very much greater where cows are affected with clinical or subclinical mastitis (Linzell and Peaker, 1972). Another period in which the mechanisms for conserving sodium fail is during prolonged diarrhoea.

Broadly, the requirements for sodium appear to be satisfied by diets containing 0.05 percent in the case of lactating ewes, 0.05 percent for beef cattle and 0.12 percent for lactating dairy cows (Aitken, 1976). Some authorities quote substantially higher figures; for example, the NRC recommends that beef cattle diets should contain 0.06 percent of Na (NRC, 1976) and that lactating cows should have 0.18 percent of Na in the diet (NRC, 1971a).

The sodium content of sorghum species is characteristically low and some values are included in Table 1. In a survey of forage sorghum in eastern Australia, 78 percent of samples were found to contain $\leqslant$ 0.03 percent of Na (Wheeler and Hedges, 1979). Smith, Middleton and Edmonds (1978) found that of 31 New Zealand forage species and cultivars analysed for sodium, the representative sorghum ranged 30th; only soybean hay contained less sodium. Ap Griffith *et al.* (1965) and Playne (1970) have shown that, although the salinity of soil may affect the absolute sodium content, the ranking of species and cultivars remains surprisingly constant. Sorghums may therefore be expected to provide a diet that is marginal or deficient in sodium in many parts of the world, particularly in inland regions. Animals are known to obtain additional sodium from their drinking water and in some cases by licking or ingesting soil, roots and bark. However, sodium supplementation is cheap and should be considered whenever animal production from forage sorghum is unexpectedly low.

As with sulphur, an outright deficiency of sodium is unlikely where the diet contains, in addition, plants other than sorghum. The weeds growing in association with the sorghum forage frequently contain much more sulphur and sodium than the sown crop (unpublished data). Jagusch *et al.* (1977) found that lambs grazing lucerne (0.035 percent of Na) containing weeds (0.35 percent of Na) gained as well as those grazing pure lucerne supplemented with salt.

**Digestibility.** High liveweight gain or high milk production per animal is impossible to sustain on forage alone unless the forage available is of moderate to high digestibility. Blaxter, Wainman and Wilson (1961) have shown, for example, that with sheep a 10 percent increase in the digestibility of a poor hay (from 50 to 55 percent) increased liveweight gain by nearly 100 percent. Of more relevance to the present discussion, at the upper end of the range an increase from 65 to 70 percent would have increased gain per day by about 25 percent.

Sorghums grow rapidly and are notoriously difficult to utilize before they mature and decline in digestibility. Ademosum, Baumgardt and Scholl (1968) reported a decline in the *in vivo* dry matter digestibility (DMD) of the cultivar SX11a from 71 percent at 52 days after planting to 57 percent at 82 days with a concurrent fall in nitrogen from 3.1 to 1.6 percent. These authors noted that although DMD values were comparable with other summer forages, voluntary intake of the sorghum was low and hence would limit liveweight gain.

Several studies of digestibility in forage sorghum have been reported although a number made use of *in vitro* techniques that were not standardized against known *in vivo* values and hence are difficult to relate to probable animal production. Although Hoveland, Anthony and Scarsbrook (1967) showed that their sorghums were comparable with alfalfa in digestibility, in general sorghum forage, at the stage when it is commonly utilized, is not of high *in vivo* digestibility. Highly digestible forage (say 70 percent DMD) can certainly be produced from sorghum × sudangrass hybrids

by a judicious combination of cutting frequency and stubble height (Fribourg, 1973). However, with the currently available cultivars this is only achieved at the expense of dry matter production. Much of the work on the interaction between management, dry matter production and *in vivo* digestibility has been conducted with the requirements of silage-making in mind. Further studies of sorghum forage management are needed to determine the management required to maximize the production of meat or milk by grazing animals. This represents an optimization of DM production/ha, high digestibility in the forage to promote high daily gain or milk production, and a forage height (and row width) that does not seriously affect utilization rates or regrowth.

Fear of HCN poisoning discourages farmers from grazing their animals on sorghum that is short, leafy and highly digestible. Brunnich (1903), a Queensland chemist, appears to have been the first to have advised farmers not to graze until the sorghum is well in flower and this advice is continued in standard veterinary texts (Blood, Henderson and Radostits, 1979). Considered solely from the viewpoint of preventing toxicity the advice is sound but were this the only criterion it would be safer still never to use sorghum for forage (Rose, 1941).

The evidence briefly reviewed here suggests that perhaps the major drawback with sorghum as a forage is not only the relatively low digestibility at the stage it is generally grazed but is also its low intake by animals. Low sodium and sulphur contents and unpalatability associated with high HCN potential all combine to depress voluntary intake of the forage. The technical problems in accurately measuring the DMD intake of animals grazing forage deficient in sodium and sulphur are considerable and no published data have been seen.

**Conclusion.** Four factors have been discussed, that alone or in combination may lead to hazardous and unsatisfactory animal production from forage sorghum. What can be done to improve animal production without recourse to substantial and usually expensive supplementation with protein and/or energy? Three measures are suggested by the information reviewed above.

*Mineral supplementation.* The sulphur available to an animal on a diet of sorghum is often marginal or, under certain conditions, frankly inadequate. Much more work is needed to define the areas of the world and the types of sorghum crop on which stock may be expected to respond to sulphur supplements. The application of gypsum and other fertilizers containing sulphur does not appear to be effective in raising the sulphur content of sorghum. Foliar application of sulphur, formulated as in fungicidal sprays to adhere to the leaves, is possible but expensive and impractical in many cases. Supplementation of the stock by the inclusion of sodium sulphate in drinking water may be considered but intakes vary greatly with the weather and would not be feasible for sheep, which drink little or nothing while grazing fresh green forage. Currently the most satisfactory method of supplementation under extensive grazing conditions, although far from ideal, is to provide salt licks containing about 10 percent of S. Where sorghum is being cut and carried to stock, sufficient elemental sulphur, sodium sulphate, or calcium sulphate and sodium chloride may be sprinkled on the feed to provide, say, an additional 8 g of S/d for a 350-kg beast. Animals should not be given or be allowed to take excessive quantities of sulphur as, in addition to a risk of hydrogen sulphide poisoning, a copper deficiency may be induced (Bird, 1970) and probably also a selenium deficiency (Whanger, 1972).

The two latter methods of supplementation have the advantage of supplying sodium as well as sulphur. A low sodium content appears to be even more common in forage sorghum than low sulphur but it is cheap to rectify; most animals can be relied upon to seek out salt supplements when they are deficient and sodium toxicity is extremely unlikely, though not unknown (Trueman and Clague, 1978).

The possibilities of providing the necessary additional minerals by mixing other plants with the sorghum are

numerous and may ensure a more uniform intake of supplementary minerals than can be achieved by means of licks. Possibilities include associations of sorghum and legumes such as cowpeas (*Vigna sinensis*) but grazing management may be required to ensure that the animals do not neglect sorghum in favour of the more acceptable species.

*Raising forage digestibility.* The digestibility of the forage on offer to animals grazing sorghum is often less than is required for high rates of liveweight gain or milk production. This is frequently attributable to the farmers' concern to obtain high dry-matter yields, the tendency of this productive species to grow faster than it can be utilized, and the farmers' desire to avoid the risks of HCN poisoning commonly believed to be inevitable in grazing while the plant is young and leafy. Successes achieved with other grasses in breeding for higher digestibility (Burton, 1970) suggest that much more could be done to improve the mediocre digestibility of sorghum cultivars currently in use. The recent commercial introduction of a cultivar that will not flower until the onset of short days is a welcome advance.

*Making risk of poisoning negligible.* The risk of HCN poisoning can be reduced to negligible proportions by minimizing the HCN potential of the forage and by ensuring that the rate of intake of cyanogenic forage is not rapid. Choice of cultivar can greatly affect the HCN potential of the forage. *Sorghum almum*, grain sorghums and "dual-purpose" sorghums, for example, tend to have higher HCN potential than sorghum × sudangrass hybrids and these in turn are higher than sudangrasses such as the cultivar Piper (Gorz *et al.*, 1977; Hedges *et al.*, 1978). Adequate fertilization will also do much to ensure a low HCN potential. In practice fatalities are uncommon. Those that do occur are, almost without exception, in very hungry animals — either stock made hungry by prolonged lack of feed as in droughts or in grasshopper (locust) outbreaks or animals with a very strong appetite drive, such as high-yielding dairy cows. Ensuring that animals are reasonably well fed before they are first introduced to sorghum is always a wise precaution. Particular attention needs to be given to this when growth of the sorghum has been slowed or inhibited by lack of moisture, cold or insect attack. On theoretical grounds animals on diets containing 0.25 percent of sulphur or more are less liable to HCN poisoning than those on diets containing little sulphur. However, experimental verification is still awaited.

*Farmers in the Yemen Arab Republic harvesting sorghum in the highland areas*

Sorghums have many agronomically desirable features (Hoveland, 1969; Fribourg, 1973) and this article has drawn attention to means whereby animal production from them can be increased, and rendered less hazardous, at little cost. However, it must not be overlooked that there are frequently alternative summer forages of comparable productivity (see, e.g., Dunavin, 1970). These may not be promoted by seed vendors, but they can provide, for regions with warm to hot summers, palatable, non-cyanogenic

*A head of a local variety of sorghum compared with an improved Egyptian variety grown on the Hodeida Agricultural Experimental Farm in the lowlands of the Yemen Arab Republic. The latter yields five to six times more.*

forage of high digestibility with adequate sulphur and sodium content (Hedges *et al.*, 1978). They include the perennial hybrid *Pennisetum americanum* × *P. purpureum* (Muldoon and Pearson, 1979) and Japanese millet (*Echinochloa utilis*), to name only two. Further improvement in animal production from sorghum species now appears to depend primarily on plant breeders giving greater emphasis to digestibility and low HCN potential among the factors to be considered (Burton, 1970) in developing new cultivars. ■

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# A new method for preserving raw milk

## the lactoperoxidase antibacterial system

**H. Korhonen**

*The article reviews the research work done on the natural, lactoperoxidase-based antimicrobial system in milk. The potential practical applications of the method described in the article do not represent the official views of FAO since, according to the Code of Principles concerning Milk and Milk Products, as declared by FAO and WHO, the term "milk" means "exclusively the normal mammary secretion obtained from one or more milkings without either addition thereto or extraction therefrom". This article should, however, be of considerable interest to those in the many developing countries that lack milk-cooling facilities, where the importance of being able to utilize a safe system of milk preservation that has advantages over existing methods will readily be appreciated.*

The collection of raw milk and its transportation to processing centres present a number of technical, economic and organizational problems in most developing countries. Many of these countries are in tropical regions and have a hot, humid climate for much of the year. Under such conditions, which are also usually characterized by low standards of hygiene, the raw milk easily spoils during storage and/or transportation. Cooling the milk is regarded as the best means of solving this problem. Facilities for this, however, are not yet generally available in many of the countries for economic and practical reasons, a situation that will, no doubt, continue for some time to come. The use of chemical preservative may, in such cases, provide the most practical alternative for the temporary preservation of raw milk. At present, the only chemical agent permitted by FAO for this purpose (and, at that, under controlled conditions) is hydrogen peroxide ($H_2O_2$) (FAO, 1957). The germicidal properties of $H_2O_2$ have been recognized for about 150 years and for preserving milk it was used for the first time nearly 100 years ago. Since then, the use of $H_2O_2$ as a milk preservative has been thoroughly investigated (Lück, 1962; Tentoni, Pastore and Ottogalli, 1968; Santha and Ganguli, 1976; Dirar and Abdel-Gadir, 1977; Santos, 1978).

*A veterinary assistant engaged in his work in a Bangladesh village.*

The $H_2O_2$ treatment of raw milk is practised in several countries, specifically in areas where, because of a hot climate and/or a long transportation period, it would be impossible, without it, to keep the milk wholesome until it reached the processing plant. The use of $H_2O_2$ has not, however, achieved general international acceptance since it involves a number of disadvantages on account of the relatively high concentrations (300-800 ppm) required to obtain the necessary effect. The main disadvantages of this treatment are:

- The changes brought about in the biochemical activity and chemical composition of the milk. These are known to affect adversely the processing properties of milk;

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TABLE 1. **The concentration of lactoperoxidase in bovine colostrum and milk**

| Sample | Average | Range |
|---|---|---|
| *Colostrum* (milking post partum)[1] | | |
| 1 | 22.8 | 11.0- 45.0 |
| 2 | 29.0 | 14.0- 53.6 |
| 3 | 52.9 | 15.1-156.0 |
| 4 | 47.7 | 14.5-140.0 |
| 5 | 37.3 | 15.1- 76.3 |
| *Milk* (14 days post partum)[1] | 20.1 | 12.8- 29.8 |
| *Milk*[2] | | |
| Healthy cows | 14.8 | 2.0- 35.0 |
| Mastitic cows | 18.3 | 6.5- 59.0 |

[1] Korhonen (1977). — [2] Korhonen **et al.** (1977).

• The possible health hazards to consumers if the milk contains residues of $H_2O_2$. However, it must be said that these undesirable effects are eliminated or are negligible if low concentrations ($<$ 100 ppm) are used (Lück, 1962).

In recent years it has been demonstrated that the additions of very small amounts of $H_2O_2$, viz., 8-9 ppm, have a substantial antibacterial effect in raw milk, providing that the milk contains roughly an equivalent concentration of thiocyanate ions ($SCN^-$) (Björck *et al.*, 1975; Reiter *et al.*, 1976). In this case, the antibacterial effect is not caused by the $H_2O_2$ itself, but by the oxidation of $SCN^-$ by $H_2O_2$, which converts it to an antibacterial compound. This reaction is catalysed by the enzyme lactoperoxidase (LP) naturally present in milk.

The application of this LP/$SCN^-$/$H_2O_2$ system, or the LP system as it is referred to in this article, as a means of temporarily preserving raw milk is at present the subject of intensive studies. It is hoped that the method will provide an improved alternative to the classical $H_2O_2$ treatment.

**Historical view.** The natural antibacterial activity of bovine milk has been known for nearly 100 years (Hesse, 1894). Over this period its importance in the keeping quality of raw milk has been postulated in numerous studies but, because of the lack of knowledge of the nature of the factors involved, this property has been neglected until recent years. It is now well-recognized that cow's milk contains several natural antibacterial systems. These include cellular factors, such as polymorphonuclear leucocytes and macrophages; humoral factors, such as antibodies, lactoferrin, lysozyme; and the lactoperoxidase/thiocyanate/hydrogen peroxide system (LP/$SCN^-$/$H_2O_2$) (Korhonen, 1973 and 1977; Reiter, 1976, 1978a and 1978b). Among these, the last system mentioned has aroused the greatest interest among those involved in the preservation of milk.

The involvement of a peroxidase in the antibacterial activity of milk was postulated by Hansen as early as in 1924. He suggested that the bactericidal properties of milk bore some relation to its oxidizing enzymes. This was not confirmed until 1958, when Wright and Tramer associated the inhibition of some strains of lactic streptococci in milk with the presence of lactoperoxidase. Portmann and Auclair (1959) identified LP with the so-called lactenin 2 that was known to inhibit the growth of certain lactic-acid bacteria in milk. On its own, however, LP has no antibacterial effect and, in 1962, Jago and Morrison demonstrated that hydrogen peroxide was also necessary for the inhibitory effect. Furthermore, they observed that certain streptococci were self-inhibited in milk because of their own $H_2O_2$ production, which activated the LP system. The third factor, thiocyanate, was identified by Reiter *et al.* in 1963.

It should be mentioned that the peroxidase-mediated antimicrobial systems, similar to that in bovine milk, have also been demonstrated in human milk, saliva and polymorphonuclear cells occurring in blood and milk (Reiter, 1978a). The biochemical mechanism and mode of action on microorganisms of these systems are at present the subject of intensive study.

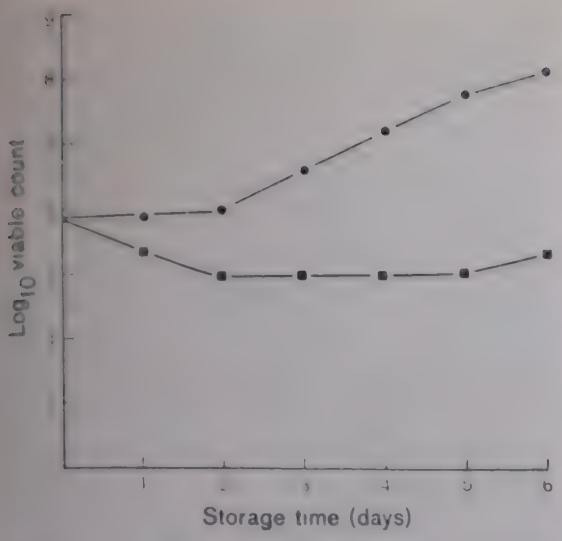
**Occurrence in milk of the components of the LP system.** The LP system is active only in the presence of all three components, viz., LP, $H_2O_2$ and $SCN^-$. This implies that in order to be active in milk it has to contain each of these factors. Furthermore, for achieving optimum activity a balanced ratio between $SCN^-$ and $H_2O_2$ content is required (Reiter *et al.*, 1976; Björck, 1977).

Concentrations of the components of the LP system naturally found in cow's milk and factors affecting them have now to be considered.

Lactoperoxidase is the most abundant enzyme in cow's milk. It is an iron-containing whey protein accounting for about 1 percent of the total whey protein content. It is characterized by its heat stability, since it retains its activity in normal pasteur-

(Above) *A village milk-collection centre in Bangladesh. Milk is delivered in unhygienic clay pots, adversely affecting the keeping quality of milk. As no cooling facilities are available, the milk has to be transported to a processing plant within a few hours.*

(Below) *A village milk-collection/chilling centre in Kenya.*




*Effect of the LP system on the psychrotrophic bacteria in raw milk stored at 5°C. The milk was supplemented so as to contain 0.25 mM $SCN^-$. ●—● = no $H_2O_2$ added, ■—■ = 0.25 mM $H_2O_2$ added at zero hours (Björck 1977).*

ization of milk (63°C/30 minutes or 72°C/15 seconds), but is destroyed at 80°C in 2.5 seconds. LP activity is found in all cow's milk, but its content varies over a wide range from almost nil up to 50 mg/l (Table 1). Several factors affect its concentration, such as breed, age and lactation stage of the cow as well as the cow's nutrition and health condition. Daily variations can also be considerable (Korhonen, 1977; Korhonen *et al.*, 1977).

The thiocyanate in milk is derived from blood in which its concentration is about 10 times higher than in milk. The $SCN^-$ content is highly dependent on the feeding regime of the cow, as many plants contain $SCN^-$ precursors. They are converted to $SCN^-$ by different biochemical transformations, such as the detoxification of cyanide, which is present in clover and enzymatic hydrolysis of glucosides, which are present in *Brassica* and *Raphanas* species (Virtanen, 1961). Levels of thiocyanate in the milk, as reported in the literature, show a wide range of variation (Table 2).

The third component, $H_2O_2$, is not present in measurable amounts in normal milk. In freshly cannulated milk it is, however, found at very low concentrations (2-4 µg/ml) (Reiter, 1976), but these may be insufficient to activate the LP system.

TABLE 2. **The concentration of thiocyanate in bovine colostrum and milk**

| Reference | Average | Range |
|---|---|---|
| *Colostrum* | | |
| Pyska (1974) | 1.27 | — |
| *Milk* | | |
| Boulangé (1959) | — | 1 - 15 |
| Virtanen (1961) | 2.0-4.0 | 0.5-8 ([1] 17.5) |
| Lawrence (1970) | 4.9 | 1.4 - 10.8 |
| Hoppe, Kozlowska and Rutkowski (1971) | 3.1-[2]5.7 | |
| Korhonen (1973) | | |
| Healthy cows [3] | 16.0 | 5.0 - 30.0 |
| Mastitic cows [3] | 21.2 | 4.0 - 59.0 |
| Korhonen *et al.* (1977) | | |
| Healthy cows | 0.90 | 0.4 - 2.2 |
| Mastitic cows | 1.25 | 0.7 - 2.8 |

[1] Maximum concentration after feeding a single dose of 6 g of $SCN^-$ salts. — [2] Average concentration after feeding rapeseed meal. — [3] Aseptically drawn milk from specially operated cows.

**Activation of the LP system in milk.** It is suggested that for the activation of the LP system in raw milk there is always sufficient peroxidase activity. The concentration necessary is only 1-2 mg/l (Björck, 1978), much less than is normally present in milk (Table 1). The factors limiting the system in milk are, therefore, considered to be the quantities of thiocyanate and hydrogen peroxide. Recent studies (Björck *et al.*, 1975; Reiter *et al.*, 1976) have shown that the antibacterial effect of the LP system is proportional to the $SCN^-$ concentration present and that maximal effect is obtained at equivalent concentrations (on a molar basis) of $SCN^-$ and $H_2O_2$. The addition of increasing equivalent amounts of these compounds results in an enhanced bactericidal effect.

Reiter *et al.* (1976) found that milk containing less than 0.015 mM (1 ppm) $SCN^-$ was not inhibitory for *Escherichia coli* in spite of the presence of $H_2O_2$. When the level was raised to 0.225 mM (≈12 ppm) the milk became strongly bactericidal for six hours and remained bacteriostatic for up to 24 hours. In whey, the bactericidal effect was even more pronounced. Similar observations have been made in regard to psychrotrophic bacteria (able to grow at 7°C or below occurring in cold-stored raw milk (see figure) (Björck *et al.*, 1975; Björck, 1977). These studies indicate that a substantial antibacterial effect brought about by the LP system can be achieved in raw milk by increasing the $SCN^-$ level up to 10-15 ppm (≈0.20-0.25 mM) and adding a corresponding quantity of $H_2O_2$. As this level of thiocyanate appears to be within its physiological concentrations in milk, it could probably easily be obtained and maintained by the suitable feeding of the cows.

The necessary $H_2O_2$ has to be supplied from an exogenous source, and this can be done by various means, such as:

- The direct addition of dilute solution of $H_2O_2$;
- The direct addition of solid peroxides, such as alkali-percarbonate, earth alkali peroxides or carbamide peroxide, all of which are compounds that release $H_2O_2$ when they come into contact with water;
- Via enzymatic production.

Enzymes used for this purpose are glucose oxidase (Reiter *et al.*, 1976; Björck, 1977) and xanthine oxidase (Björck, 1979). The latter is of considerable interest as a possible *in vivo* source of $H_2O_2$, since milk contains large amounts of this enzyme.

In laboratory experiments all these methods have proved to be capable of activating the LP system resulting in a considerable bactericidal effect in both milk and semi-synthetic media.

As the LP system involves three components in a well-balanced ratio it can be expected that it will be sensitive to interferences by a number of substances occurring in milk. Although the knowledge of the presence of such substances is incomplete it seems that, in practice, they do not interfere to such an extent that the antibacterial effect is greatly diminished. Substances involved may include various peroxidase inhibitors, catalase and reducing agents having free sulphydryl (—SH) groups.

In the case of peroxidase inhibitors, high concentrations of $H_2O_2$ (i.e., 300-500 ppm) inactivate the lactoperoxidase in milk. The antibacterial effect is thus due to $H_2O_2$ itself which is by nature different from the LP system.

Catalase competes with lactoperoxidase for the hydrogen peroxide and may, at low concentrations of the latter, reduce the bactericidal effect (Reiter *et al.*, 1976). At normal physiological levels of LP and catalase in milk no interference by catalase has been observed. This suggests that LP has a higher affinity than catalase for $H_2O_2$, and it explains also why the system is effective against catalase secreting bacteria. It may be concluded therefore that the LP system will function also in mastitic milk in spite of its higher catalase content. This, however, remains to be proved.

Reducing agents such as cystein and glutathione have long been known to reverse the antibacterial activity of the LP system (Wright and Tramer, 1958). Milk proteins contain very little free —SH groups. Those present are located mainly in β-lactoglobulin and do not react with oxidizable substances in unheated milk. Interference by reducing agents is therefore likely to be of minor importance in raw milk. In heated milk, however, the —SH groups are freed and presumably in this case the LP system would not appear to be active as in raw milk.

**Mechanism of the LP system.** Considerable knowledge of the chemical nature of inhibitory compounds involved in the LP system has accumulated in recent years. Earlier studies (see reviews by Reiter, 1976; 1978b) have suggested that the active agent is an intermediate oxidation product of $SCN^-$. There is now substantial evidence that the antibacterial compound is hypothiocyanate ($OSCN^-$) (Hoogendoorn *et al.*, 1977; Thomas and Aune, 1978). It is suggested that this compound is formed and decomposed in the following reactions:

$$SCN^- + H_2O_2 \xrightarrow{(LP)} OSCN^- + H_2O$$

$$4\ OSCN^- + H_2O \longrightarrow 3\ SCN^- + SO_4^{2-} + CO_2 + NH_4^+$$

$OSCN^-$ produced in the above reaction oxidizes, at the bacterial cell surface, certain vital enzymes, which contain —SH groups. This reaction results in the immediate inhibition of the respiration of the cell. Depending on the bacterial strain concerned, it will lead to the temporary growth-inhibition or death of the cell. The mode of action of the active agent on different bacteria is not as yet known in detail. It has been demonstrated that in the case of *E. coli*, the oxygen uptake and active transport of glucose and various amino acids is stopped immediately after exposure to the system. The death of the organism occurs, however, after about two hours' exposure to the system (Reiter, 1976; 1978b).

Most of any excess $OSCN^-$ not reacting with bacteria reverses spontaneously to $SCN^-$ within a few hours, but it is partially decomposed also into sulphate, ammonia and carbon dioxide, leading to a loss of antibacterial activity. The $H_2O_2$ added to the system is fully consumed in the course of reaction.

These characteristics bear some important implications when the LP system is being utilized in the preservation of raw milk. They will be discussed later in this article.

**Antibacterial spectrum of the LP system.** The activity of the LP/$SCN^-$/$H_2O_2$ system in milk or synthetic media has been established against a wide range of bacteria. This range involves micro-organisms that are im-

TABLE 3. **Antibacterial activity of the LP/$SCN^-$/$H_2O_2$ system in milk or in a synthetic medium against different bacteria**

| Group/bacterial species | Nature of effect | Reference |
|---|---|---|
| Starter cultures | | |
| *Streptococcus cremoris* | Bacteriostatic | Wright and Tramer (1958) |
| | | Jago and Morrison (1962) |
| | | Reiter, Pickering and Oram (1964) |
| Thermophilic lactobacilli | Bacteriostatic | Portmann, Gaté and Auclair (1962) |
| Mastitis organisms | | |
| *Streptococcus agalactiae* | Bacteriostatic | Reiter, Pickering and Oram (1964) |
| | | Mickelson (1966) |
| *Streptococcus uberis* | Bacteriostatic | Reiter, Pickering and Oram (1964) |
| *Staphylococcus aureus* | Bactericidal | Reiter and Bramley (1975) |
| *Escherichia coli* | Bactericidal | Reiter and Bramley (1975) |
| Pathogenic and enteropathogenic organisms | | |
| *Salmonella typhosa* and *S. paratyphi* | Bactericidal | Hansen (1924) |
| *Salmonella typhimurium* | Bactericidal | Reiter *et al.* (1976) |
| *Escherichia coli* | Bactericidal | Reiter *et al.* (1976) |
| *Pseudomonas aeruginosa* | Bactericidal | Reiter *et al.* (1976) |
| *Streptococcus pyogenes* | Bactericidal | Mickelson (1966) |
| Milk-spoilage organisms | | |
| *Pseudomonas fluorescens* | Bactericidal | Björck *et al.* (1975) |
| Gram-negative strains | Bactericidal | Björck *et al.* (1975) |
| Raw milk flora | Bactericidal/ Bacteriostatic | Björck (1978) |

*Sampling for milk quality control at a village milk collection centre in Bangladesh. Simple tests for hygienic and chemical quality are applied to each milk delivery.*

portant both from the point of view of the keeping quality of raw milk and the manufacture of fermented milk products and also in human and animal health. Table 3 groups such bacteria according to their significance.

Although the LP system is considered as non-specific in regard to antibacterial activity, considerable differences in sensitivity are found between different bacteria. Depending on the bacterial species or strain of the same bacterium the effect can be bactericidal or bacteriostatic. Among streptococci, even resistant strains have been detected (Reiter, 1976). A clear distinction has also been found in the sensitivity of Gram-positive and Gram-negative bacteria, the former generally being only temporarily inhibited while the latter are killed.

*Milk reception at a village milk collection/chilling centre in Kenya. Evening milk is chilled and kept over night in cans, which are immersed in a chilled water bath. If the cooling facilities are not sufficient, the milk may become sour before reaching the milk plant next morning. This could be avoided with a controlled use of an appropriate milk preservation system.*

Most Gram-positive organisms, e.g., lactic-acid bacteria, are deficient in catalase and therefore the peroxide produced metabolically by them is accumulated in the growth medium. This $H_2O_2$ can activate the LP system, provided LP and $SCN^-$ are present, and this leads to the self-inhibition of bacterial growth. Certain cheese starter cultures probably are inhibited in milk in this way, causing failures in cheese making. Most lactobacilli and streptococci are only temporarily inhibited and not killed by the LP system. The single species of streptococci known to be killed is *Streptococcus pyogenes* (Group A), the infective agent of scarlet fever (Reiter and Bramley, 1975). This organism is rapidly killed in raw milk suggesting that the LP system is naturally active against it.

Most Gram-negative bacteria possess catalase activity, which decomposes continuously any metabolically produced $H_2O_2$. These bacteria, therefore, are not self-inhibited in milk through the LP system. The $H_2O_2$ necessary to complete the antibacterial system has to be supplied by an exogenous source, as described earlier. Recent studies (Björck *et al.*, 1975; Reiter *et al.*, 1976; Björck, 1978) have shown that the LP system activated in milk, whey or synthetic media by any of these means is bactericidal to a number of Gram-negative organisms that are of medical and hygienic interest (Table 3). Based on these observations, the possibility is now being explored of utilizing the LP system in the prevention of bovine mastitis and gastrointestinal infections of calves and piglets (Reiter and Bramley, 1975; Reiter, 1978b).

Of special interest in this connection is the finding that the LP system is strongly bactericidal for *Pseudomonas* species and other psychrotrophic bacteria isolated from milk (Björck, 1978). These organisms are known to cause spoilage when growing in refrigerated raw milk, thus considerably limiting its storage time and use for processing purposes.

**Preservation of raw milk by the LP system.** The fact that the LP system is active against a great variety of micro-organisms usually contaminating milk suggests that it can be utilized to prevent the bacterial spoilage of raw milk, irrespective of whether the milk is cooled or not, under conditions where long storage and transportation periods are unavoidable. This idea is supported by the results from laboratory and field trials so far carried out. The efficiency of the LP system in extending the storage time of refrigerated raw milk has clearly been established in laboratory experiments simulating farm conditions (Björck, 1978). Activation of the system in milk, i.e., increasing the $SCN^-$ level to 0.25 mM and adding the equivalent amount of $H_2O_2$, resulted in a substantial reduction of the bacterial flora and prevented the multiplication of psychrotrophic bacteria for up to five days (see figure). A similar effect was also achieved when batches of milk, supplemented so as to contain 0.25 mM $SCN^-$, were pooled daily and stored at 5°C. The LP system was activated on the second and fourth day of storage by the addition of hydrogen peroxide only. After six days' storage this milk had a bacterial number some 1 000 times smaller than the untreated control.

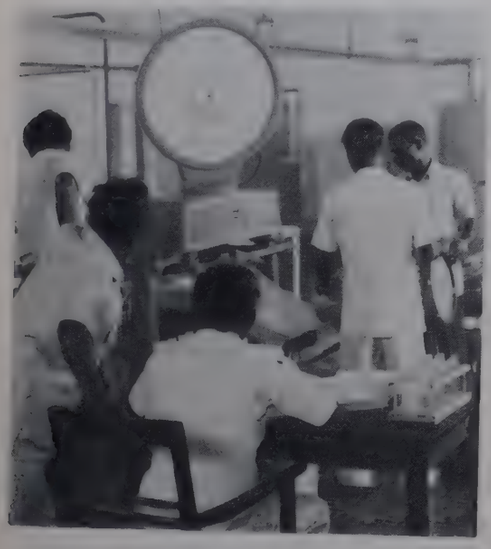
It is noticeable that this treatment does not affect the physico-chemical properties of milk, such as rennet coagulation time, content of free fatty acids, growth of starter cultures and/or the number of bacteria surviving pasteurization (Björck, 1978). This makes the LP system superior to the classical $H_2O_2$ treatment which causes distinct changes in the processing properties of the treated milk.

Although the LP system may also be used in the extension of the storage time of cooled raw milk, it can be expected to be most useful in its application in the storage of raw milk under conditions where cooling facilities are not available.

This view is substantiated by labo-

ratory experiments that indicated that also at 30°C the LP system is capable of developing a bacteriostatic, antibacterial effect in milk for seven to eight hours (Björck, 1979). Such an effect could be sufficient to prevent the raw milk from deteriorating during its transportation from a collection centre to the processing plant. At the present time, trials are being carried out in a tropical country to evaluate the efficiency and applicability of the LP system for the preservation of raw, uncooled milk under practical field conditions. Preliminary results so far obtained (Björck, 1979) are promising, and suggest that this method could provide an improved alternative to the $H_2O_2$ treatment. Further trials, in which comparison of the two methods can be made, will be required to confirm this view.

*Milk reception at a modern milk-chilling centre in Bangladesh.*



**Health aspects of the LP system.** As the LP system, in order to be active in milk, necessitates small additions of $H_2O_2$ and $SCN^-$ the health aspects involved in this treatment have to be clarified before approval of the use of the system is likely to be given by any country. The facts at present available indicate that the antibacterial agent produced by the LP system is completely destroyed by heating the milk at 60°C for 15 minutes (Björck *et al.*, 1975). Conventional pasteurization will therefore ensure that the antibacterial effect is eliminated before the milk is further processed or distributed to consumers.

Because the small amount of $H_2O_2$ needed is fully used up in the course of the reaction, the residual effect of this treatment will in practice result in $SCN^-$ ions in the milk. The levels required, i.e., 10-15 ppm, are, however, within the ranges normally found in the milk, but are low compared with the concentrations occurring in human saliva (50-300 ppm) (Densen *et al.*, 1967) or in human gastric juices (40-50 ppm) (Ruddell, Blendis and Walters, 1977). The potential risk to human health of the residual thiocyanate levels seems, therefore, to be very small. A prerequisite for the utilization of this method in raw milk preservation is the provision of a safe and reliable method of ensuring that exact and uniform dosaging of the system's components into the milk takes place. This point will need to be given special attention in further studies.

In general, there is some evidence that the LP antibacterial system present in milk, saliva and the intestinal tract acts as a natural biological defence system (Reiter, 1978a and 1978b). Should this hypothesis prove to be valid, the utilization of the LP system as a milk preservative, provided it is done properly, is not likely to be the cause of any health hazards.

**Conclusions.** The experience so far gained suggests that the lactoperoxi-

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*A road tanker being loaded with chilled milk at a large collection/chilling centre in Bangladesh.*

dase catalysed antibacterial system provides a new biochemical method for the preservation of raw milk. It might be utilized under conditions where cooling facilities are lacking or limited, transportation distances are long, and where an extended storage time for cooled milk is required. Although the system seems to be naturally active in raw milk against certain bacteria, its practical application requires its activation through additions of small amounts (up to 10 ppm) of thiocyanate and hydrogen peroxide. The preservative effect achieved is comparable to classical $H_2O_2$ treatment but the LP system appears to have many distinct advantages over that system.

The main difference is that considerably smaller amounts (40-100 times less) of $H_2O_2$ are needed. Because of this the LP system does not appear, unlike the $H_2O_2$ treatment, adversely to affect the physico-chemical and nutritional properties of milk. The potential health risks from possible residual $H_2O_2$ are also, practically speaking, negligible and no subsequent chemical treatment (such as with catalase) is needed to eliminate excess $H_2O_2$. The one factor requiring consideration in this respect is the potential toxicological risk from the increased thiocyanate levels. More field trials are needed in which the efficiency and applicability as well as the economic aspects of the LP system can be evaluated and comparisons of it made with the $H_2O_2$ treatment. Finally, it must be stressed that the LP system, as is also the case of other systems utilizing other chemical preservatives or methods of cooling, is not a substitute for clean milk production or for proper heat treatment of milk at the dairy plant. ■


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# Embryo transfer in cattle breeding

**I. Wilmut**

*For many years cattle breeders have been able to use artificial insemination* (AI) *to select the best bulls and so improve their herds. The availability of techniques to recover embryos from selected donors and transfer them into unmated recipients would enable them to select more intensively among the females in their herds and so improve their herds more quickly. The purpose of this review is, first, to describe the methods that are being developed for recovery, manipulation and transfer of embryos, and then to discuss their practical application.*

**Superovulation of donors.** In order to increase the potential yield of embryos it is first necessary to induce the donor animal to have several ovulations. The most commonly employed gonadotrophin is pregnant mares' serum gonadotrophin (PMS), which is a glycoprotein with a very long half-life in cattle (Schams *et al.*, 1977) so that one injection is effective in stimulating follicle growth over a period of several days. The injection of PMS is commonly given on day 16 of a normal oestrous cycle or on any day between day 8 and day 15 if an injection of prostaglandin is given 48 hours later to induce luteal regression (day 0 = day of oestrus). The majority of animals are in oestrus approximately 96 hours after the injection of PMS. It is commonly accepted that animals being induced to superovulate should be inseminated more than once with several doses of semen. Laboratories vary in their practice but may employ two or three inseminations, with a total of six or nine standard doses of semen being used for each donor. It is not known why several inseminations are required. The most striking characteristic of the response to this treatment is the enormous variation. It is common experience that ovulation rate in response to the same treatment may vary from 0 to over 40. Typical results are shown in the chart. A list of factors believed to influence the response to PMS is given below:

Dose of PMS
Batch of PMS
Breed of cow
Individual cow
Individual oestrous cycle
Day of cycle when treated
Nutrition
Season

Dose response curves for PMS have been established in cattle (e.g., Mauléon *et al.*, 1970). It is considered that an optimum dose exists, below which the total ovarian response is less than maximal, and above which the response tends to be more and more in the form of unruptured follicles.

Different batches of PMS have been considered to have different true potency; however, in the most careful study carried out so far the difference was not statistically significant (Stewart, Allen and Moor, 1976). It seems that any differences that do exist are small compared with other sources of variation.

Application of the same treatment to a variety of breeds of cattle produced mean responses in the most responsive breed almost four times that in the breed with the poorest response (Saumande *et al.*, 1977). It is not known whether or not all breeds have maximum response at the same dose of PMS.

Apart from these factors the single greatest source of variation is that between individual cows and even between repeated applications of the treatment to the same cow.

Three factors that influence the response of an individual are nutrition (in sheep, Allison, 1975, 1977), season (Saumande *et al.*, 1977) and the day of the cycle on which treatment is initiated (Phillippo and Rowson, 1975).

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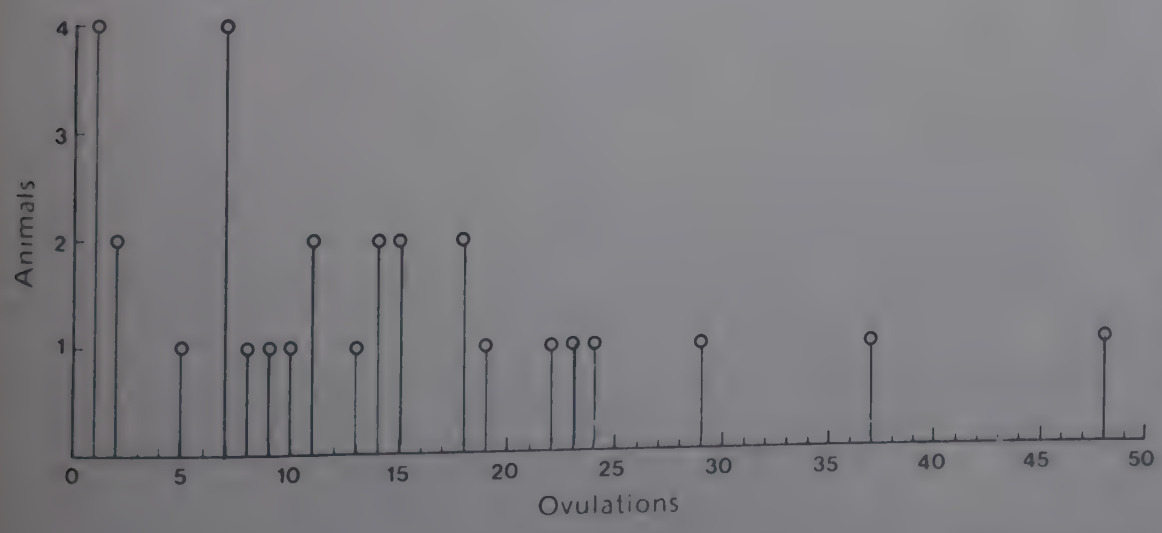
TABLE 1. **Number of normal and atretic follicles in individual cows slaughtered on days 8-14 of the cycle, by diameter**

| Greater than 4 mm | | | Greater than 3 mm | | |
|---|---|---|---|---|---|
| Normal | Atretic | Total | Normal | Atretic | Total |
| 1 | 3 | 4 | 3 | 9 | 12 |
| 1 | 1 | 2 | 12 | 9 | 21 |
| 12 | 8 | 20 | 23 | 14 | 37 |
| 1 | 2 | 3 | 2 | 8 | 10 |
| 5 | 1 | 6 | 30 | 21 | 51 |
| 3 | 8 | 11 | 16 | 23 | 39 |
| 3 | 9 | 12 | 10 | 18 | 28 |
| 1 | 10 | 11 | 4 | 16 | 20 |
| 2 | 1 | 3 | 2 | 2 | 4 |
| 9 | 1 | 10 | 20 | 6 | 26 |
| 1 | 8 | 9 | 4 | 12 | 16 |
| 1 | 11 | 12 | 16 | 36 | 52 |

**Source:** Rajakoski, 1960.

These observations indicate that there is some factor that varies greatly between and within animals during the time that treatment is being applied. It seems probable that variations in the follicle population lead to the variation in response. Follicles that are in the early stages of atresia can be stimulated to further development by injection of PMS (Hay and Moor, 1975) and it seems probable that the follicles that ovulate in response to PMS are some of those which at the time of the injection are normal or are in the early stages of atresia and 3 mm or more in diameter. Table 1 shows the number of normal and atretic follicles greater than 3 mm in diameter in cattle killed on days 8-14 of the cycle. It can be seen that the variation in follicle number is comparable to the variation in number of ovulations (as shown in the chart). Certainly the time taken for growth of follicles is a great deal longer than the interval from PMS injection to ovulation, thus setting a limit to the potential number of ovulations (see Turnbull, Braden and Mattner, 1977, for data in sheep).




*Number of ovulations per animal for 30 Charolais cows following a single treatment with 2 000 IU* PMS *and 0 or 1 500 IU human chorionic gonadotrophin* (HCG)

There is no evidence of marked individual variation in the rate of metabolism of PMS (see McIntosh, Moor and Allen, 1975, for data in sheep). This implies that variations in ovarian response to PMS or in follicle population at the time of injection are the major determinants of the number of ovulations.

In considering the possibility of increasing the average number of ovulations or of making the response more uniform, three ideas should be considered. First, as PMS is a complex molecule with a very long half-life in cattle, the hormone is still detectable in the peripheral plasma of superovulated donors at the time of embryo recovery some 10 days later (Schams *et al.*, 1977), and pituitary gonadotrophins have been used instead of PMS because of their very short half-life (e.g., Seidel *et al.*, 1977a). Although in some experiments the response has been improved (e.g., Seidel *et al.*, 1977a), the extra labour of twice daily injections may limit the use of these hormones. Nevertheless the question remains: is there a more effective gonadotrophin than PMS? Second, ovulation rate has been increased by active immunization of sheep against androstenedione (Scaramuzzi, Davidson and Van Look, 1977) and this suggests that interference with androgen levels may alter ovarian or pituitary response. Can one use this approach to improve the response to superovulation? Finally, if one knew what controls the development of follicles to the stage at which they respond to PMS, it might be possible to devise treatments that artificially increase the number of follicles available.

**Embryo recovery.** The surgical methods of embryo recovery had the disadvantages that they were expensive and could cause infertility by creating adhesions in the body cavity. These methods have now been replaced by non-surgical procedures that depend on passing equipment into the uterus through the cervix. A Franklin three-way catheter used for this is shown in Figure 1.

The three-way catheter has the advantage that fluid can flow continu-

ously through the uterine horn so that it is possible to use smaller volumes of fluid to recover the embryos. The disadvantage is that almost inevitably the instrument will be slightly larger and so more difficult to pass through the cervix in heifers. As all of the catheters are flexible, some form of support is necessary in order to pass the cervix. This support can take the form of a stilette within the catheter or a metal sleeve that has first been introduced along a steel rod. While the sleeve is more effective, again it inevitably increases the overall diameter of the equipment at the cervix.

No direct comparison of the two methods has been made, but it seems reasonable to conclude that in experienced hands either can be very successful (see Table 2). Variation between groups probably reflects variation in ovulation rate far more than differences in efficiency.

TABLE 2. Yield of embryos by non-surgical recovery

| Reference | Embryos per cow (number) | Cows (number) |
|---|---|---|
| *Two-way catheter* | | |
| Seidel *et al.*, 1977b | 7.7 | 28 |
| Brand *et al.*, 1977 | 3.1 | 72 |
| Greve and Lehn-Jensen, 1977 | 3.1 | 29 |
| *Three-way catheter* | | |
| Newcomb, Christie and Rowson, 1977 | 7.6 | 21 |

**Non-surgical transfer.** The most commonly employed method of non-surgical transfer is the Cassou gun (Figure 2) as used for artificial insemination. The embryo is loaded into a straw within 0.1-0.2 ml of fluid. The straw is placed in the gun and the sterile plastic sheath applied. The vulva and vagina are cleaned and the gun passed through the cervix and along the uterine horn. The distance that the gun is passed has varied from 0-15 cm beyond the bifurcation of the uterine horns. The highest conception rates are apparently obtained by those who deposit the embryo a relatively short distance beyond the bifurcation (e.g., Renard, Heyman and Mesnil du Buisson, 1977). Conception rates obtained following transfer of one embryo per recipient

**Figure 1.** *Non-surgical recovery equipment showing Franklin 3-way catheter with cuff inflated and steel introducer*

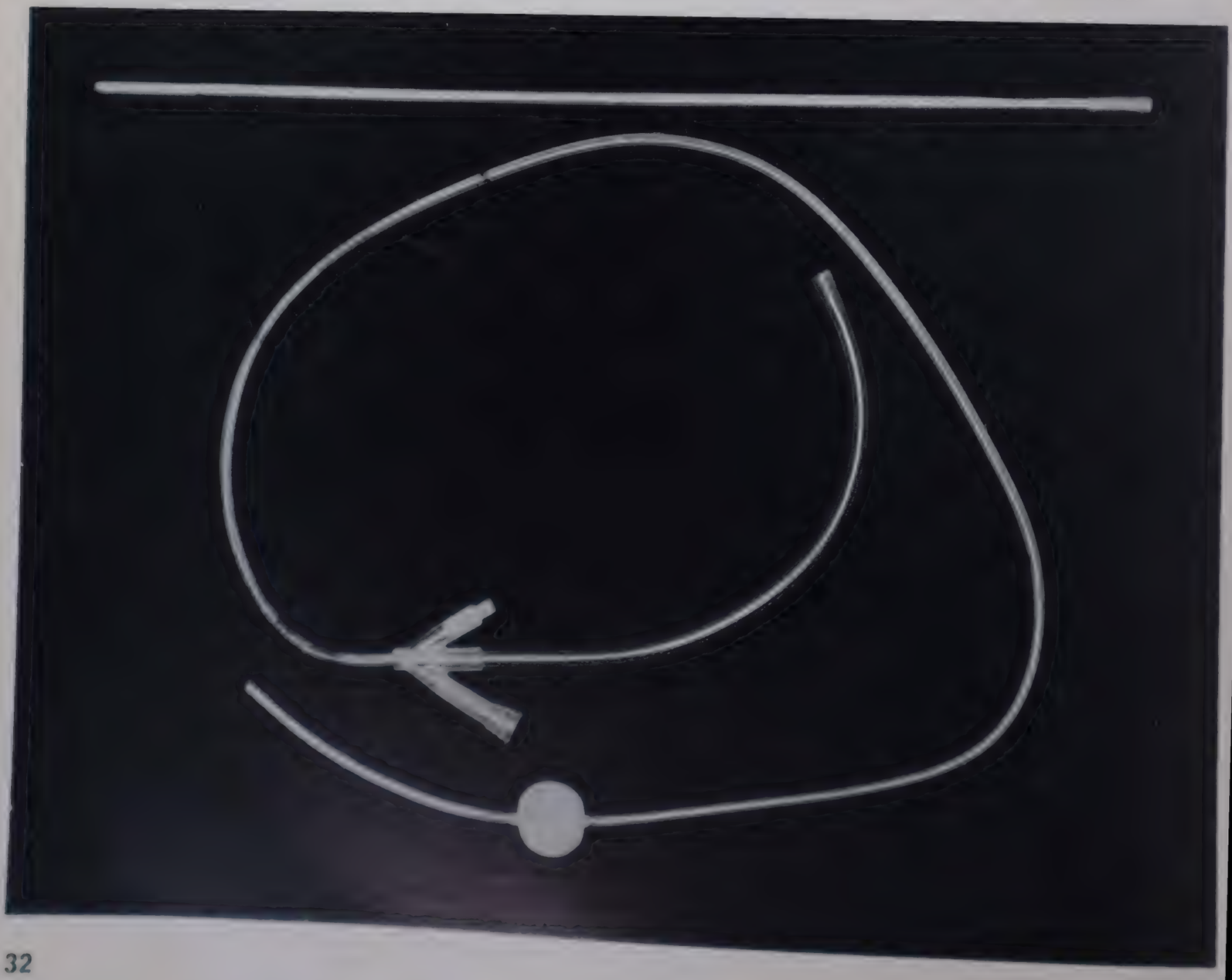


**Figure 2.** *Cassou gun as used for non-surgical transfer and artificial insemination. The straw can be seen between the metal gun and the plastic sleeve.*

with the Cassou gun vary enormously (from 20 to 50 percent) and while some of this variation may be due to differences between groups of animals it is clear that we do not understand the basic reason for the failure to achieve the conception rates achieved by surgical transfer (e.g., 55 percent, Church and Shea, 1976).

Non-surgical transfer of an embryo into the contralateral horn of a mated animal does not reduce conception to the mating, however low the survival rate of transferred embryos (e.g., Sreenan, 1978). Therefore, the failure of transfer cannot reflect a systemic mechanism following trauma of the cervix or uterus. Use of a site of transfer some distance away from the normal position of an embryo at that time is not responsible for poor conception as transfer to the tip of the uterine horn with a flexible apparatus produced fewer pregnancies than use of a Cassou gun (I. Wilmut, 1977 — unpublished). It also seems unlikely that introduction of bacteria with the gun or active expulsion of embryos are the cause of failure (Brand *et al.*, 1976a and b). The most likely explanation is that a trauma is caused locally at the site of transfer, and that this in some way disrupts embryo development.

**Freezing of embryos.** A great deal of progress has been made since the first calf was obtained from a frozen embryo (Wilmut and Rowson, 1973) and methods are now available by which a majority of embryos survive freezing and thawing (Willadsen, 1977). The method that is most convenient to use employs 1.5 M dimethyl sulphoxide (DMSO) added at room temperature and a cooling rate of 0.3°C per minute from seeding temperature to between —30° and —36°C. After a period of slow cooling (0.1°C/min) or holding at an intermediate temperature for 10-30 minutes the embryos are plunged into liquid nitrogen. It is not clear which method of thawing is most effective (Willadsen, 1977). Similar results have been obtained by Smorag and his colleagues at Balice (personal communication).

While considerable progress has been made in simplifying the cooling and warming procedures, it is still unfortunately necessary to use high concentrations of DMSO, which must be removed before transfer (Willadsen, Polge and Rowson, 1978). Further research is necessary before a breeder can thaw and transfer embryos directly on his farm in the same way he can use semen at present.

**Sexing of embryos.** It has long been known that the sex of embryos could be established by preparation of a karyotype using cells removed from the trophoblast (McFeely, 1966) and in recent years the technique has been successfully applied in cattle (Hare *et al.*, 1977). The method involves culture of the tissue with colcemid to arrest a large number of cells at metaphase, dissociation of the cells, disruption of the cells by use of hypotonic solutions before fixation, spreading and staining of the chromosomes. Only a large proportion of the embryos can be sexed successfully (54 of 90, i.e. 60 percent) and failure to establish sex seemed to be associated with embryos from some donors (Hare *et al.*, 1977). The subsequent conception rate is probably only slightly lower than in control situations.

This technique undoubtedly has research applications, but is too laborious to be of significant commercial value. Practical application of a sexing procedure depends upon development of a simpler method, perhaps involving detection of the H-Y antigen on embryonic tissue (Krco and Goldberg, 1976).

**Practical application.** Use of the techniques described allows the col-

lection of embryos from chosen superior cows, before transfer into recipient cows, with the possibility of storing the embryos in the meantime. In considering the application of embryo transfer it is necessary to define the objective, to determine the cost of employing embryo transfer and finally to compare this cost with the cost of alternative methods of achieving the same objective. It is essential that the procedures being considered should be as simple as possible as each additional procedure will cause some further loss of embryos.

Applications of the techniques so far have been limited to multiplication of those breeds whose number of animals in each case in a particular country was very small, so that each calf was extremely valuable and, more recently, to multiplication of valuable pedigree stock. There can be little doubt that the breeders whose animals command the highest prices will use embryo transfer to increase the number of surplus stock available for sale. The impact of this application on the national herd will be very small.

Embryo transfer can also be used in several ways to enhance breed improvement schemes. It can be used, for example, to increase selection intensity of bull dams by producing the requisite number of bulls from fewer dams, and it can be used within a herd to increase the rate of improvement. Several possibilities have been considered in detail for both dairy and beef cattle (e.g., Land and Hill, 1975; Hill and Land, 1976; Krausslich, 1976; Nicholas, 1977; Petersen, 1978; Wilmut and Hume, 1978).

Precise conclusions will not be possible until several analyses have been made of the costs of obtaining particular levels of genetic improvement. Some indications can, however, be given.

It is possible to use embryo transfer within herds to obtain more replacements from the best animals. If, for example, a cow has a yield 3 000 litres above the herd average, it is tempting to think of obtaining extra calves from her rather than from her lesser contemporaries. The amount a producer can afford to spend on each transfer has been calculated for circumstances in the United Kingdom (Wilmut and Hume, 1978). If a heritability of 25 percent is assumed, then daughters of such a cow will have yields some 375 litres above the average for daughters of all cows. This extra milk can be valued at UK £ 20 per lactation and it is then necessary to set against the total extra income from several lactations the interest paid on money spent at the time of transfer some three years before the first increase in income is generated. If a breeder is trying to obtain heifers, the justifiable investment in each transfer is much less than the extra value of each calf because not all embryos survive, and half the calves will be bulls. As estimated for the United Kingdom in January 1978 the justifiable investment in each transfer was UK £ 9 plus the cost of AI.

As the cost of non-surgical transfer is much greater than this amount it is evident that embryo transfer should not be used in dairy cattle unless calves can be sold for considerable sums. The conclusion is exactly the same for beef cattle.

If availability of acceptable cows to be used as bull dams in dairy breeds is a serious practical problem, as it is in some countries, or in breeds that have only a small number of animals, the production of adequate numbers of bulls by embryo transfer may be extremely useful. Alternatively, additional bull calves may be tested for a beef characteristic to enhance the dual-purpose character of the breed (e.g., Krausslich, 1976; Petersen, 1978). The effect of the schemes then depends upon the accuracy of the assessment and the degree of inbreeding that is considered to be acceptable (Petersen, 1978).

Detailed consideration has also been given to the possibility of improving beef cattle, and it appears the rate of improvement can be increased dramatically (Hill and Land, 1976). The schemes depend upon intensive selection of both heifers and bulls after performance-testing of a beef characteristic.

In general, it seems that breeders will not be able to recover investment made in embryo transfer from the increased production of milk or meat, but will have to sell animals at high prices. Bulls produced in such schemes could improve the national herd by use of AI, but the economic and social difficulties in establishing test schemes and obtaining access to desirable animals may limit the development of the necessary selection and transfer schemes.

Embryo transfer can also be employed to create twin pregnancies (e.g., Rowson, Lawson and Moor, 1971). It is undoubtedly true that this procedure would increase biological efficiency by producing more meat from the same amount of grass, however the economic value is not as clear (Wilmut and Hume, 1978). Application will certainly depend upon producers having confidence that 12 months from the time of transfer the value of each calf will be high (Wilmut and Hume, 1978). Furthermore, it is important that further research be carried out on the nutrition of twin-bearing cattle and their smaller calves.

The ability to freeze embryos increases the convenience of any transfer, but freezing will kill some embryos. In contrast, freezing is almost essential for the export of embryos. Export of frozen embryos after quarantine may be cheaper than shipping adult animals, and has the advantage that calves born to "native" mothers will acquire passive immunity to local diseases from the mothers' colostrum. Freezing has other research applications, already discussed (Wilmut, 1976).

**Conclusion.** Embryo transfer is particularly useful in four situations. First, the improvement of beef cattle after performance-testing of both heifer and bull calves. The practicability of such a scheme will depend on the degree of control exerted by the breed society or government on breed improvement or upon the existence of very large herds. The cost of such a scheme can only be justified to the breeder by the high price obtained for extra calves sold after such a transfer or by governments prepared to pay for increases in national efficiency. Second, the export of frozen embryos offers a very economical and

efficient method of establishing valuable breeds or lines in different parts of the world — provided that the skill required to handle the embryos is available. Third, the production of bull calves from selected dams will be a matter of some convenience to AI centres, although the genetic benefits may be small. Finally, the establishment of twin pregnancies by embryo transfer will probably increase biological efficiency, but cannot be justified unless the producer is confident of high calf-prices for at least a year.

Although the large-scale commercial application of the techniques is not justified at present the situation will change as techniques are improved or if food prices increase. We are only just beginning a new era. ■

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# Protein enrichment of starchy substrates for animal feeds by solid-state fermentation

**J.C. Senez, M. Raimbault and F. Deschamps**

In spite of difficulties resulting from present economic conditions, the large-scale industrial production of single cell proteins (SCP) will undoubtedly develop soon in industrialized regions and countries, such as western Europe, Japan and the USSR, in which the development of new protein sources is becoming an absolute and urgent necessity.

*A priori*, one could also expect the SCP industry to make a decisive contribution to the problem of hunger in the Third World. To this, however, there are several major obstacles.

To be economically viable, an SCP production unit should have a minimum capacity of at least 100 000 tons per year, corresponding to a capital cost of 50 to 70 million dollars. However, a plant producing 100 000 tons of SCP from paraffins would require an equal supply of substrate and should thus be associated with an oil refinery having a minimum capacity of about 3 to 5 million tons of crude protein per year. Similar considerations apply to the production of SCP from natural gas or from methanol. Such facilities are obviously absent in most non-oil-producing developing countries of Asia, Africa and Latin America. Moreover, these countries may not have a potential market nor an appropriate transportation and distribution network for the commercialization of 100 000 tons of SCP per year.

Clearly, countries that cannot at present import food or feeds because of currency shortages will also not be able to import industrial SCP from abroad. Consequently, it is of the utmost importance that they should be able to develop their own protein resources. Besides hydrocarbons and methanol, a wide variety of raw materials potentially utilizable for SCP production could be considered. However, most of these are available at too high a cost to be economically competitive or are available in quantities too small for protein production on a really significant scale. Among the substrates in supply and at the right price, special emphasis is usually put on cellulosic materials. Up to the present, however, despite many attempts made to use these materials, little success has been achieved, the main difficulty being the lack of cellulolytic organisms with an adequate growth rate.

**Figure 1.** *Pilot fermenter (5201) for protein enrichment. This picture shows the attached probes (P, pH) and device for water spraying. The fermenter is set up on a balance for control of the water content of the material.*

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In contrast, starchy materials and, more specifically, cassava in the tropical regions or potatoes in more temperate climates, are of obvious interest. This is because of their high productivity per hectare and their excellent rate of conversion to biomass by a great number of fast-growing microorganisms.

In order to be economically competitive, the production of protein from starch should not be undertaken by classical fermentation in liquid medium, under aseptic conditions, followed by biomass separation and drying. As in the case of SCP production from paraffins or methanol, the maximization of such sophisticated technology would require a minimum production well over the potential market available to most developing countries, and would result in high investment and operation costs. Moreover, in developing countries, the collection, transportation and storage of large quantities of raw materials would lead to major difficulties. Because of these considerations, one is led to adopt quite a different approach consisting of protein enrichment of starchy material by a simplified technology that can be applied at the farm or village level, and that would thus simultaneously combine the cultivation of the raw material, its conversion into protein and its direct utilization for animal feeds. Economically, a great and decisive advantage of such an integrated procedure would be that it would prevent the intermediary profit-taking and speculation that would inevitably arise if either the raw material or the product were commercialized.

To be feasible at the rural level, a protein-enrichment process should not require aseptic conditions and it should be possible to perform it in a single operation. Furthermore, the final product must be sufficiently rich in protein to be utilizable as such, without the need for a secondary concentration step. This last requirement introduces a biotechnological difficulty that was responsible for the failure of many previous attempts to achieve direct protein-enrichment of starchy materials. In a mash of raw material dense enough to be directly utilized for animal feeding, the major problem is to maintain aerobic conditions and oxygen-transfer efficiency so as to prevent anaerobic contamination of the culture.

TABLE 1. **Protein enrichment of cassava by solid-state fermentation**

| | |
|---|---|
| *Initial substrate* (g) | |
| Cassava flour [1] | 100 |
| $SO_4(NH_4)_2$ | 9 |
| Urea | 2.7 |
| $PO_4KH_2$ | 5 |
| Water | 100-120 |
| *Optimal growth conditions* | |
| t° | 35-40°C |
| Initial pH | 3.5 |
| Inoculum (spores/g of flour) | $2 \times 10^7$ |
| Incubation time (hours) | 30 |
| *Composition of the product* (percentage) | |
| Protein [2] | 18-20 |
| Residual sugars [3] | 25-30 |
| Water [4] | 68 |

[1] Carbohydrates: 90 percent; protein; 2 percent; water: 8-9 percent. — [2] Determined by the Lowry method, percentage of the dried product. — [3] Determined by enzymatic hydrolysis (amyloglucosidase) and Somogyi-Nelson titration, percentage of the dried product. — [4] Percentage of the wet product.

*Tempeh*, a food prepared in Indonesia by treating soybeans with the fungus *Rhizopus viygae*, and many other food preparations obtained by solid-state fermentation of soybeans or other materials with filamentous fungi (Martinelli and Hesseltine,1964; Hesseltine, 1965; Gray, 1970) are traditionally used in various parts of Asia and Africa but these fermentations do not increase the protein content of the initial materials. However, procedures for direct protein enrichment of cassava by liquid fermentation (Reade and Gregory, 1975; Gregory *et al.*, 1976) or solid-state fermentation (Brook, Stanton and Wallbridge, 1969) have been described, although protein enrichment by the solid technique did not exceed 3-4 percent, which is insufficient to enable use to be made of the material as a complete feedstuff. The liquid process with fungi gave rise either to technical or to sanitary problems.

TABLE 2. **Protein enrichment of various raw materials**

*Percentage dry matter*

| Material | Initial composition | | Final product | |
|---|---|---|---|---|
| | Protein | Carbohydrate | Protein | Carbohydrate |
| Cassava | 2.5 | 90 | 18 | 30 |
| Banana | 6.4 | 80 | 20 | 25 |
| Banana waste | 6.5 | 72 | 17 | 33 |
| Potato | 5.0 | 90 | 20 | 35 |
| Potato waste | 5.0 | 65 | 18 | 28 |

A new procedure for solid-state fermentation (Raimbault and Germon, 1976) that overcomes the problems described above has been developed in France. A preliminary report of this technique was presented at the 5th International Conference of the Global Impacts of Applied Microbiology (Raimbault *et al.*, 1977).

**Laboratory investigations.** This new procedure is based on the homogeneous distribution of spores and mineral salts in the mass of the starchy substrate used in a suitable form. The preparation of a porous, granulated material with adequate pH, temperature and moisture content is essential to ensure good aeration and the fast growth of mycelium all through the mass.

Thus, the coarsely ground raw material, containing 30-35 percent moisture, is maintained at 70-80°C for 10-15 minutes by gently steaming so as to gelatinize the starch granules. After cooling to 40°C, the preparation is mixed with water containing the inoculum (spores), the nitrogen sources (ammonium sulphate and urea) and potassium phosphate, and brought to a moisture content of 55 percent. By mechanical stirring, the inoculated substrate spontaneously takes the form of well-separated and uniform granules some 2-3 mm in diameter.

The initial substrate, optimal growth conditions and composition of the product required in the protein enrichment of cassava or other starchy materials are summarized in Table 1. This method has already been used with a variety of starchy materials, including cassava, whole potatoes, potato wastes from industrial starch-works and banana refuse. The results reported in Table 2 show that, after 30 hours' incubation, a product containing, on average, 20 percent

true proteins (measured by the Lowry method) and 25 percent residual reducing-sugars is obtained. The rate of conversion of carbohydrates to protein is 20 to 25 percent.

Up to the present experiments have been performed with a selected strain of *Aspergillus niger* having high amylolytic activity and a suitable amino acid composition. However, it should be pointed out that many other filamentous fungi, particularly among strains traditionally used in Asian countries for human consumption, have been successfully tested by this technique. This method does not require aseptic conditions, since the selective growth of the mould results from acidic pH, low moisture content or heavy spore inoculation. Microscopic examinations of products indicate that all spores germinate after 6-8 hours and that during the growing phase all the mycelia are developing with no production of conidia. At the end of the fermentation no spores could be observed. Bacteriological examination of fermented products indicate neither pathogens nor significant development of anaerobic bacteria. The aerobic microflora remains at the same level during the first 20 hours, after which the number of aerobic bacteria quickly decreases.

**Experimental pilot scale-studies.** From laboratory results, new equipment was designed for this solid-state fermentation process (Deschamps and Meyer, 1979). All the operations were conducted in a commercial bread-making blender modified for the purpose (Fig. 1).

Steaming or aeration were performed by passing steam or air through the perforated bottom of the tank. A control system using conventional probes was designed to keep suitable pH, moisture and temperature conditions by stirring the product and spraying it with water or mineral solutions. This control system was monitored by a temperature sensor as soon as the temperature reached the set point. The pH, temperature and regulation time could thus be monitored to provide a simple check of the growth rate and harvest time.

With the organism at present utilized, the optimal temperature is 40°C, but some growth still takes place from 30 to 45°C without a significant change in the final protein yield. The initial moisture content is critical, with an optimum of 55 percent. In the course of fermentation, the water content is progressively increased to a final value of 70-75 percent.

The patterns of reactions involved in a solid-state fermentation process on potato waste are illustrated in Figure 2, which shows the production of protein, reducing sugars and water content as well as the pH of the preparation. The curve marked by crosses is of special interest, since it shows that, during a total incubation time of 30 hours, the monitored devices for mechanical stirring and spraying had to operate for only 5 hours, thus demonstrating the excellent efficiency of the cooling device. Furthermore, it needs remarkably little power to operate, a fact of obvious importance in regard to production costs of solid-state fermentation and its economical feasibility at the village level in tropical regions.

TABLE 3. **Agro-economic prospects**

| Item | Cassava | Soybeans [1] |
|---|---|---|
| *Productivity of raw material and protein* | | |
| Raw material (tons/ha) | 40 | [2] 1.8 |
| Moisture content (percentage) | 70 | — |
| Proteins (tons/ha) | [3] 1.8 | 0.6 |
| *Conversion into animal product (pig)* [4] | | |
| Alimentary conversion rate | | 3:1 |
| Protein consumption (kg) | | |
| Birth to weaning [5] | | 11.3 |
| Weaning to slaughter [6] | | 25.5 |
| Total [7] | | 36.8 |
| *Overall agro-economic prospects* | | |
| Protein productivity per hectare, protein-enriched cassava compared with soybeans (appx.) | 3:1 | |
| One hectare of cassava can produce, by solid-state fermentation, enough protein for the feeding of approximately 50 pigs | | |

[1] 34 percent protein. — [2] Data from USDA. — [3] For 20 percent protein enrichment, and 25 percent lost in dry matter during fermentation. — [4] From C.A. Shacklady, **Proteins from hydrocarbons**, New York, Academic Press. 1972. p. 115-128. — [5] Birth to weaning: 70 days; + 25 kg; diet with 15 percent protein. — [6] Weaning to slaughter: 130 days; + 85 kg; diet with 10 percent protein. — [7] Total: 200 days; 110 kg.

TABLE 4. **Optimal productivity of protein-rich feeds**

| Feed | Total yield (tons/ha) | Protein Content (%) | Protein Yield (tons/ha) |
|---|---|---|---|
| Soybeans | 1.8 | 34 | 0.6 |
| Rapeseed | 3.0 | 23.3 | 0.7 |
| Sunflowers | 2.5 | 22 | 0.55 |
| Horse beans | 3.2 | 28 | 0.9 |
| Peas | 3.0 | 25 | 0.75 |
| Protein-enriched cassava | 9.0 [1] | 20 | 1.8 |

[1] 40 tons per hectare of cassava, with 70 percent moisture content, 25 percent lost during fermentation, dry weight.

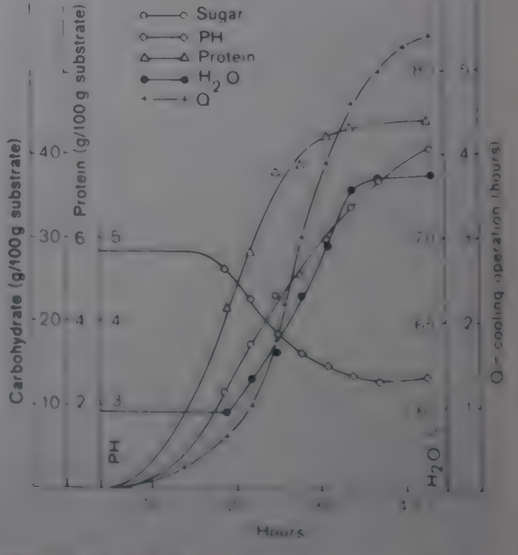


**Figure 2.** *Solid-state fermentation of potato waste showing the production of protein, reducing sugars and water content as well as the pH of the preparation.*

Present studies on this solid-state fermentation process are actively being developed in France by ORSTOM and IRCHA,[1] in close collaboration with the starch industry in order to obtain economic benefit from potato wastes. The scaling-up of the process to a fermenter unit of 1-$m^3$ and 250-kg (dry matter) capacity has been undertaken and is in progress. This equipment, which is expected to be operative soon, will be utilized for large-scale nutritional and toxicological testing on certain classes of livestock (pigs and poultry), for further optimization of substrate preparation and growth conditions and, finally, for the determination of actual investment and operations costs. It is intended to extend the experimental work to the setting up of experimental production units in tropical Asia and Africa, in order to adapt the procedure to local climatic and agro-economic conditions.

**Agro-economic perspectives.** As has already been pointed out, the two main potential sources of starch available for protein enrichment are cassava in tropical regions and potatoes in temperate climates. Protein enrichment of cassava is of special interest in those semi-arid regions of Latin America and Africa where climatic conditions are not suitable for the cultivation of soybeans or other protein-rich feeds.

The productivity of cassava per hectare varies widely from one region to another depending on climatic and agro-technological conditions. The yield of roughly 16 tons (harvested weight) per hectare in northeastern Brazil, for example, can easily be increased by the use of fertilizers and by improved agricultural methods to 40 and even 60 tons per hectare. Other advantages of cassava are its low production costs, easy storage in the soil for several months and the fact that it is also an excellent source of calories for feeding.

On the basis of a productivity of 40 tons per hectare and 20 percent protein enrichment using solid-state fermentation, cassava or potatoes may provide 1.8 tons of protein per hectare, i.e., the amount required for the feeding of 50 pigs (Table 3). This is about three times the quantity of protein per hectare provided by soybean cultivation in the United States. The crop-yield and protein productivity per hectare of other protein sources conventionally utilized for animal feeding, are shown in Table 4.

TABLE 5. **Compared productivity and gross product per hectare**

| Feed | Average yield (tons/ha) | Current price [1] (US$/ton) | Gross product (US$/ha) | Comparative gross product |
|---|---|---|---|---|
| Maize | 6 | 82.9 | 497.4 | 114 |
| Wheat | 5 | 127.7 | 638.5 | 147 |
| Soybeans | 1.8 | 241.8 | 435.2 | 100 |
| Protein-enriched cassava | [2] 9 | [3] 97.1 | 873.9 | 200 |

[1] As of 29 September 1978. — [2] Cassava: 40 tons per hectare, with 70 percent moisture, 25 percent dry matter lost by fermentation, dry product containing 20 percent protein. — [3] Estimated from current price (US$ 213.6, cif. Rotterdam) of soybean meal with 44 percent protein, for the protein content alone.

From recent prices (October 1978) and from the average yields of agricultural products, one can compare the gross product per hectare of maize, wheat, soybeans and protein-enriched cassava. The figures given in Table 5 strikingly demonstrate the economic value of protein enrichment by solid-state fermentation. In fact, in the case of cassava, the value of the residual sugars (35 percent dry weight) should increase the gross product figure. However, for a rural community combining the production of a raw material with protein enrichment and its direct utilization for animal feeding, the actual gross product should be estimated, not from the commercial value of protein, but from the value of the feed produced. Moreover, as already pointed out, one of the major agro-economic advantages of protein-enriched cassava is the possibility of producing balanced feed in regions where no other suitable source of conventional feed protein is available.

Quite obviously, to be economically competitive, the process of protein enrichment by solid-state fermentation depends ultimately on the investment and production costs of the process. It would be premature at this stage to make a firm calculation in this regard since it is necessary to wait for the results of pilot operations carried out at the farm level. However, in the present state of technological development, and from the data reported in Table 5, it may be confidently predicted that the process will prove to be a valuable one. ■

[1] ORSTOM = Office de la recherche scientifique et technique outre-mer, 24, rue Bayard, 75008 Paris, France; IRCHA = Institut de recherche en chimie appliquée, 18-bis, Boulevard de la Bastille, 75012 Paris, France.

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# Grazing cattle under coconuts

**S.G. Reynolds**

Considerable attention has recently been drawn to the need for the integration of livestock and tree crops, particularly coconuts (Thomas, 1978). Since the total area of the world under coconuts is estimated to exceed 5 million hectares, the potential area available for this form of integrated agriculture is extensive.

While it can be argued that first priority should be given to food crops, with livestock as a secondary consideration, it is suggested that at least two clear-cut situations exist, with intermediate situations in between, which justify the integration of livestock and coconuts.

First, where population densities are high, available land areas are small and priority has to be given to the growing of food crops, cattle can be introduced to utilize various crop by-products and forages cut and hand-carried to tethered or stall-fed animals. This system is traditionally widespread in Southeast Asia, and recent work in Mauritius, Seychelles and elsewhere (Preston and Leng, 1978; Preston, 1979) with banana, sugar cane, sweet-potato vines and *Leucaena leucocephala* suggests that intensive beef and milk production are possible by smallholders on farms of 2-4 hectares on which food and cash crops are integrated with livestock, and manure is utilized for biogas production before being recycled to the land.

*Hereford steers on common guinea grass* (Panicum maximum) *with centro* (Centrosema pubescens)

Second, where pressure on land resources is not critical and where more extensive intercropping is possible, the choice of intercrops may range from cocoa and coffee to the grazing of cattle on local or improved pastures. Too often the land between the coconut palms under such conditions is covered with unproductive weeds, and there is a need for much better utilization of such land in order to increase overall returns per hectare.

This second aspect of the subject and specifically the grazing of cattle under coconuts, is the subject of the present article. By-product utilization has already been discussed in a series of papers by Preston and others (Anon., 1977).

## Traditional use of cattle under coconuts

Traditionally, cattle have been run as "sweepers" or "brushers" to keep indigenous grasses and weeds under control on large plantations, so as to enable the easy location of fallen nuts. The meat produced by the cattle was regarded as something of an unexpected bonus, in addition to the benefits derived from the main coconut crop.

## The case for intercropping coconuts with grazed pastures

*Spacing.* The spacing of coconut palms in many areas is such that intercropping is necessary fully to utilize the available growth potential of the crop. In many coconut-growing areas much of the land is under-utilized and the dense cover of weeds and bushes not only reduces nut production by competing for available water and nutrients but makes it physically difficult to find the nuts once they have fallen.

*Land quality.* In many areas coconuts cover land of good quality close to settlements. Expensive land-clearance schemes are often proposed for poor-

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quality highland areas when, in fact, a maximization of production on the better-quality lands by the use of limited inputs for intercropping makes better economic sense than bringing into production at a low level, and at considerable cost, an additional large area of mainly poor quality land.

*Coconut yields.* These may receive a better boost as a result of clearing undergrowth from existing areas and better management (thus raising production per hectare) than by planting large areas of new trees which, under poor management, may have low productivity.

*Labour shortage.* Where there is a shortage of labour or where co-operative societies may choose to utilize labour in other activities then the running of cattle under coconuts may be the most economic alternative in those areas not required for food crops.

*Plantation activity.* On a plantation scale a system of paddocks grazed in rotation by cattle can be incorporated into routine plantation activities with complementary benefits to both.

*Soil fertility.* The establishment of legume-based pastures leads to increased soil fertility. Where fertilizer is applied to the intercrop the main crop will also benefit.

*A weed-infested stand of coconut*

## Special considerations

The aim of intercropping should be to increase overall returns per hectare and this usually involves maximizing returns from both or all crops. In selecting pasture species it is necessary to consider characteristics such as productivity, palatability, persistency, nutritive value, pest and disease resistance and adaptability to local soil and climatic conditions. In addition there are a number of special factors that have to be taken into consideration to ensure that the system has a chance of success.

*Competition.* The successful integration of cattle and coconut production requires that both the main crop and the intercrop are considered as important components of the system. In the choice of pasture species their effects on coconut-palm productivity are important. In most cases, under both young and mature stands of coconut, there is sufficient light, moisture and nutrients to encourage the growth of weeds. Intercropping aims to replace the weeds with a second crop that is of economic value. The factors available for growth will determine the absolute level of production, but the choices of intercrop and the system of management will determine the relative contribution of each to the total production per unit area. If the intercrop competes too strongly with the main crop then its high yields may be achieved at the expense of the main crop. In the final analysis the aim must be to achieve a moderately high level nutrient-cycling equilibrium that is maintained by the digestive system of the cattle, which facilitates the rapid turnover of nutrients from the herbage (augmented possibly by occasional

*Thin Hereford steers used as weed "sweepers" on local pastures under coconuts*

*Establishing* Brachiaria brizantha *in herbicide-treated strips in local pasture*

small inputs of fertilizer) back into the soil organic matter (Javier, 1974).

*Shade tolerance.* Coconut intercrops must be adapted to lower light-intensities than do crops under open conditions. Degree of light intensity will vary with the spacing and age of the coconuts, being lowest in closely spaced plantations and where coconuts are between 8 and 20 years of age. In selecting pasture species it is important to use those able to utilize most efficiently, and to the maximum benefit, the reduced light. The range of light conditions can be summarized as follows: pre-bearing trees (<5 years) — good light-transmission; young trees (5-20 years) — poor light-transmission; mature trees (>20 years) — increasingly good light-transmission. For these different light conditions different pasture species may be recommended. Where light transmission values fall much below 40 percent then both production values and the range of species adapted to much greater shade are severely reduced (Table 1).

*Trampling and soil competition.* Animals may cause damage to soil structure if they are grazed on heavy soils under very wet conditions and if very high stocking-rates are maintained. Good herd management with periodic cultivation are necessary requirements on such soil, requirements, incidentally, that are equally necessary with nontree crops.

*Coconut harvesting.* Where nuts are harvested directly from the trees no change in harvesting policy is required. Where nuts are collected from the ground, collection is made easier if the rotation of cattle is synchronized with nut collection so that nuts are collected from a grazed paddock as soon as cattle are moved to a new paddock.

*Cattle damage to young trees.* If pastures are established or native pasture is grazed when coconut trees are still short (and especially if they are less than five years old), then trees may suffer physical damage from the grazing cattle, particularly in the form of chewed and damaged fronds, which, in severe cases, may kill the young tree. Experimental work on cattle repellants to protect small coconut-palms from browsing cattle has generally proven inconclusive.

TABLE 1. **Grass species and percentage light transmission**

| | Light transmission values | | | |
|---|---|---|---|---|
| | <40% | <50% | 50-75% | >75% |
| *I. indicum* | → | | | |
| *B. humidicola* | → | | | |
| *B. dictyoneura* | → | | | |
| *D. aristatum* | → | | | |
| *D. caricosum* | → | | | |
| *S. dimidiatum* | → | | | |
| *S. secundatum* | → | | | |
| *A. compressus* | → | | | |
| *B. brizantha* | | → | | |
| *B. decumbens* | | → | | |
| *P. maximum* | | → | | |
| *B. miliiformis* | | → | | |
| *P. maximum* cv. Embu | | | → | |
| *B. ruziziensis* | | | → | |
| *?B. mutica* ... | | | → | |
| *?B. decumbens* | | | | → |

*Collecting coconuts from a well-grazed pasture*

*Tree spacing.* Because of the decrease in light transmission resulting from a decrease in the spacing of coconut trees it is suggested that 8 × 8 m is about the minimum for spacing between palms where the economic establishment of improved pastures is being considered (Guzman and Allo, 1975). At higher palm-tree densities the cattle-carrying capacity is considerably reduced.

## Pasture species

A wide range of different grass species has been reported in early investigations carried out in Sri Lanka, the West Indies and East Africa, and in more recent work in Southeast Asia and the Pacific region (Gutteridge and Whiteman, 1978; Guzman and Allo, 1975; Reynolds, 1978a and b; Santhirasegaram, 1966).

Although grass species like napier (*Pennisetum purpureum*), and tall guinea grass (*Panicum maximum*) have performed well, in general they are regarded as too aggressive in their competition with the coconut palms for moisture and nutrients. Furthermore, their erect growth habit makes location of fallen nuts difficult.

Generally speaking, grass species most suited to the reduced light conditions under coconut palms 10-40 years old are sod-forming stoloniferous grasses that form short to moderate height swards, provide moderate carrying capacity, do not compete excessively with coconut production and allow fallen nuts to be located easily, are cheap and easy to establish from cuttings and compete well with aggressive weed species. Such grasses include Angleton grass or Alabang X (*Dichanthium aristatum*), batiki (*Ischaemum indicum*), cori (*Brachiaria miliiformis*), koranivia (*B. dictyoneura* or *B. humidicola*), palisade (*B. brizantha*), signal (*B. decumbens*), and possibly creeping guinea (*Panicum maximum* cv. Embu). Although para (*B. mutica*) is popular in the Philippines, experience in the Pacific has shown that it requires good management under the high light conditions of old plantations (trees 50-60 years old), or where wide spacing has been used. In Zanzibar, pemba grass (*Stenotaphrum dimidiatum*) and, in other coastal areas of Africa and the Pacific islands, St. Augustine grass (*S. secundatum*), are widespread under coconut plantations.

The legumes most suited to coconut plantations include centro (*Centrosema pubescens*) and siratro (*Macroptilium atropurpureum*), with puero (*Pueraria phaseoloides*) and sometimes calopo (*Calopogonium mucunoides*) used as pioneers. Legumes that combine well with *B. brizantha* and *B. decumbens* include hetero (*Desmodium heterophyllum*) and alyce clover (*Alysicarpus vaginalis*). Sensitive plant

(*Mimosa pudica*) should be utilized where it is indigenous, but needs to be controlled. Leucaena (*Leucaena leucocephala*) can be grown as a double-row hedge (rows 1 m apart) between every two rows of coconuts.

A factor that, along with shade, may have an important bearing on the selection of grass and legume species is the level of management available. A grass such as *B. miliiformis* with centro, and regularly fertilized, will give excellent yields with a well-managed dairy herd, while *I. indicum*, *D. aristatum* or *S. dimidiatum* with *D. heterophyllum* or *A. vaginalis* are more suited when beef steers are being grazed under minimal levels of management and the use of low inputs (Table 2).

TABLE 2. **Selected grasses and legumes, and level of management**

| Level of management | | |
|---|---|---|
| Low | Moderate | High |
| Grasses | | |
| *I. indicum* | *B. brizantha* | *B. miliiformis* |
| *S. dimidiatum* | *B. decumbens* | *B. mutica* |
| | *B. dictyoneura* | *B. brizantha* |
| | | *B. decumbens* |
| Legumes | | |
| *D. heterophyllum* | *C. pubescens* | *C. pubescens* |
| *A. vaginalis* | *M. atropurpureum* | *M. atropurpureum* |
| | *D. heterophyllum* | *D. intortum* |
| | *L. leucocephala* | *L. leucocephala* |

## Management practices

*Establishment.* Initial clearing of weeds and bushes can be achieved using beef animals as weeders and tramplers followed by hand cutting or chemical treatment of trees and stumps. Further preparation for planting can be done by disc plough or chisel plough (ploughing depth should be limited to 15-20 cm), or herbicide spraying with a range of costs and quality of soil preparation depending on whether the pasture is to be established from cuttings or seed. Because of the cost involved in purchase or collection of seeds it is considered that establishment of grass cuttings (compared with seeds or divided rootstocks) is the most practical method in situations where cost of labour is low or where cuttings can be disced into the soil. Thus the emphasis on mainly stoloniferous grass species. Legumes such as *D. heterophyllum* may also be established from cuttings but in the case of centro and siratro local seed production can provide seed for sowing with hand spinners.

Where coconut trees are more than 20 years old and well-spaced, pastures are readily established. If coconut trees are between 10 and 20 years old and well-spaced then the amount of light is the critical factor. Where light transmission is between 40 and 55 percent then grasses like *B. brizantha, B. decumbens, B. dictyoneura, B. miliiformis* and *I. indicum* should be considered. Between planting and about 10 years of age (and especially in the period 0-5 years) young coconut trees may suffer physical damage from grazing cattle. When establishing coconuts, therefore, it is probably best to raise catch crops of vegetables, root crops, pineapples, etc., for four years before the pastures are established in the fifth year. Cattle can then begin to graze the pastures as the first nuts appear and as the trees grow out of reach of the cattle, thus reducing the likelihood of physical damage to tree fronds. Judicious fertilization of young coconuts should ensure rapid growth and allow for earlier grazing since the fronds will grow out of reach of the animals that much earlier. If catch crops are not grown then a cover crop of puero or calopo would protect and enrich the soil between the young coconuts and serve as a valuable pioneer legume in which grass and other legumes can later be established.

Although young stock might be introduced earlier it is probable that cattle should not graze under coconuts younger than about 5 years old. An application of 125 kg/ha of superphosphate will encourage early legume establishment. Full grazing may be possible within six months of establishment where cuttings are planted thickly in moist soil.

*Cattle grazing* B. miliiformis *pastures under coconuts*

*Management.* Careful, early grazing, good weed control, followed by good stock control, especially of grazing pressure and stocking rate, should ensure that a pasture provides a good return on the resources invested in its establishment. Suggested stocking rates are shown in Table 3 for beef steers on pastures on volcanic soils of moderate to good fertility in Western Samoa under coconuts spaced at 9.1 m on the square giving 50 percent light transmission. A convenient generalization used in this case for grasses like *B. brizantha, B. decumbens, B.*

*miliiformis*, etc., was that stocking rates could be increased by approximately 0.1 animals per ha for every increase of 5-6 percent light transmission above 50 percent. In general, the adoption of a rotational grazing system has been found to be the best in practice to allow for weed control, fertilizer application, legume control, tick control and nut collection. If a pasture degrades rapidly then destocking or reduced grazing pressure may be necessary or the fertilizer application may be increased in order to raise the level of nutrient circulation so as to match nutrient loss.

## Effects of pastures on coconut yields

Work in Sri Lanka reported by Ferdinandez (1972) and in Western Samoa has shown that grass species vary in their ability to depress copra yields, with aggressive species like guinea grass causing much greater yield depressions than grasses like *B. brizantha* and *B. miliiformis*. However, in the most tropical areas with the recommended species and adequate fertilizing and moisture for both coconut and intercrop, no such yield depression occurs and copra yield increases of 10-20 percent have been reported.

TABLE 3. **Suggested stocking rates for beef steers on pastures in Western Samoa**

| Pastures | Steers/ha [1] | ha/steer |
|---|---|---|
| Poor local pasture | 0.25 | 4 |
| Good local pasture | 1.5 | 0.7 |
| *B. brizantha* et al.[2] | 2.0-2.5 | 0.5-0.4 |
| *P. maximum* | 2.5-3.5 | 0.4-0.3 |

[1] Steers in 225-360 kg liveweight range gaining 0.25-0.5 kg/head/day, coconuts spaced 9.1 m × 9.1 m. — [2] B. brizantha, B. dictyoneura, B. decumbens, B. miliiformis. P. maximum cv. Embu.

## Economics

It is now generally accepted that, for economic reasons, coconut lands should be intercropped, with the possible exception of areas of hybrid coconuts. Where there is a choice of intercrop it is undoubtedly true that intercrops of cocoa, coffee, banana, etc., and multi-storied cropping systems (Nelliat, Bavappa and Nair, 1974) will produce much higher returns than grazing cattle. No comparison of these systems is presented here.

Various studies have been conducted in the Philippines comparing the economics of coconuts grown in monoculture with coconut areas and improved pastures grazed by cattle. The overall profitability of coconut and cattle integration has been established (Anon., 1978) in studies by Barker and Nyberg (1968) and Guzman (1970, unpublished) and by Manciot and Mandret (1976) in the New Hebrides in the Pacific. Walton (1972) emphasized that the major economic advantages of introducing cattle to coconut plantations were reduced weeding costs, increased copra production from a better rate of recovery of fallen nuts, and income from beef sales. A more detailed study conducted in the Solomon Islands by Carrad (1977) showed that, where local pastures were grazed by cattle, nut pickup increased from approximately 75 percent to over 90 percent. He concluded that grazing cattle under coconuts can be a profitable alternative to the production of copra alone.

Work in Western Samoa may be used to demonstrate the likely costs of establishment and returns from improved pastures under coconuts (see Tables 4 and 5). Although evaluation of establishment costs is complicated by the wide range of initial ground and vegetation conditions, the cost of hiring equipment, clearing, cultivating,

*Weighing cattle from a trial comparing weight gains on different grass species under coconuts*

*Establishing stoloniferous grasses by hand under coconuts*

TABLE 4. **Costs per hectare of pasture establishment and maintenance under coconuts, Western Samoa**
*(US$)*

| | Materials | Labour | Total |
|---|---|---|---|
| *Pasture establishment* [1] | | | |
| Mean | 30 | 98 | 128 |
| Range | 26-33 | 72-124 | 105-151 |
| *Pasture maintenance* [2] | 15 | 20 | 35 |

[1] First year costs. — [2] Costs in second and succeeding years.

planting, fertilizing and weed control in the first year is approximately US$ 128/ha, with weed control and fertilizer costs in succeeding years at about US$ 35/ha. These costs compare with an estimate for establishment costs of US$ 90 for the Philippines by Guzman and Allo (1975). While these costs may seem high the returns from improved pastures are also high when compared with local pastures. Trials showed that Hereford steers grazing *D. aristatum* produced an extra 92 kg/ha/year of meat compared with local pastures with steers on grasses like *B. brizantha* and *B. miliiformis* producing over 130 kg/ha of extra meat. At US$ 1.05/kg this represents an extra income of from US$ 96 to 153/ha/year.

*A* Brachiaria brizantha - Leucaena leucocephala *pasture under coconuts six months after establishment*

These data were derived from areas of improved and local pastures where fencing and watering facilities and costs were similar.

Of course, the extra return from copra would be in addition to this figure.

TABLE 5. **Value of extra beef produced on improved pastures, Western Samoa**

| Grass | Extra [1] beef (kg/ha/year) | Value [2] (US$/ha/year) |
|---|---|---|
| *I. indicum* | 92 | 97 |
| *B. brizantha* | 130 | 137 |
| *B. miliiformis* | 146 | 153 |

[1] Compared with good local pastures. — [2] Sale price of beef US$ 1.05

## Conclusions

While the debate about which crop to establish under coconuts will undoubtedly continue, there are strong economic reasons for advocating some form of intercropping with coconuts. In many areas the establishment of grass legume pastures under coconuts using one of the stoloniferous grass species named above, together with controlled grazing, would represent a considerable improvement on the present practice of allowing weeds and bushes to grow with coconuts in monoculture. Grazing cattle on improved pastures under coconuts should result in extra income from the sale of meat and milk and increased returns from the sale of copra, thus increasing the income/ha for the farmer. ■

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# NEWS AND NOTES

### Second Venezuelan Congress of Animal Science

The second Venezuelan Congress of Animal Science will be held from 19 to 22 October 1980 at the Ezequiel Zamora University. The event is sponsored by the University and several Venezuelan science and animal science associations. Working groups will be established for genetics and physiology, nutrition and forages, socio-economics, and industrial processing and health. Participants are invited to present papers following the format established by the Latin American Association of Animal Production (ALPA). These should be sent before 31 July 1980 to Dr G. Rios R., Programa de Producción Agricola Animal, Universidad "Ezequiel Zamora", Guanare, Portuguesa, Venezuela.

### World congress on sheep and beef cattle breeding

This congress will be held in New Zealand from 28 October to 13 November 1980. Important deadline dates: deposit of NZ$ 250 by 15 July 1980; balance of tour costs and registration fee by 15 September 1980; and summaries of contributed papers by 15 July 1980.

### Fourth International Karakul Symposium

The above Symposium, originally scheduled for October 1980 in Berlin has been postponed and will be held at a time and place to be announced in due course.

### Association for the Development of the Meat Institute (ADIV) of France

At the request of the French National Bureau of Scientific and Technical Information (MIDIST) the above Association has recently announced that it is to publish, every two months, a review of the principal developments and events that have occurred in the meat industry, as well as in the food industry when they concern matters of interest to the meat industry. The review will include articles of a technical and economic nature at a technically high level by specialists in the industry.

The object of the review is to assist the industry in keeping up with worldwide developments in order to improve its competitiveness in French and foreign markets.

The Association will also publish a monthly newsletter containing general information and short technical articles.

Further information can be obtained from ADIV, 24, Avenue des Landais, BP 45, 63170 Aubière, France.

### Ninth international course on dairy cattle husbandry

The ninth international course on dairy cattle husbandry will be held from 10 March to 12 June 1981 at Wageningen, the Netherlands. The course is at postgraduate level and is intended to refresh the knowledge of participants and to provide them with new information on important aspects of dairy cattle husbandry.

The closing date for applications is 1 December 1980. Information can be obtained from the International Agricultural Centre, Post Box 88, 6700 AB Wageningen, the Netherlands.

### Auction of transferred embryos in cows

During recent years there has been considerable progress in the development of techniques for recovering embryos from selected donors for transfer into unmated recipients. As a result, more intensive selection can be undertaken in herds, thus effecting improvement more rapidly.

As a final step in the commercialization of the technique the first auction in Europe of cows pregnant with embryos transferred from outstanding dams bred to superior sires was held at Reading, United Kingdom on 31 October 1979. Twenty-five pregnant animals were presented. The highest price paid for an embryo was US$ 10 000, the average price was US$ 3 300 and in addition US$ 792 was paid for each of the carrier animals.

# PUBLICATIONS

### Indian Journal of Animal Genetics and Breeding

The first issue of this journal was published early in 1979. It is the official publication of the Indian Society of Animal Genetics and Breeding, which was organized in 1978. The journal publishes original research papers and invites critical reviews on animal-population genetics, animal cytology and cytogenetics, biochemical and immunogenetics, molecular genetics, behavioural genetics and animal breeding.

The journal is at present published bi-annually (January and June). Membership of the Society is open to all persons actively engaged and professionally interested in animal genetics and breeding. The annual fee for members outside India is US$ 10. There is an entrance fee of US$ 1 for new members.

Enquiries regarding manuscripts can be made to Dr R.M. Acharya, Editor, Indian Journal of Animal Genetics and Breeding, and to the Director, CSWRI, Avikanagar, via Jaipur (Rajasthan) 304 501, India. Enquiries regarding subscriptions and business matters should be directed to Dr D.K. Biswas, Secretary, Indian Association of Animal Genetics and Breeding and Joint Commissioner (Poultry), Ministry of Agriculture and Irrigation, Dept. of Agricultural Research and Education, Krishi Bhavan, New Delhi-110001, India.

### Proceedings of the International Symposium on Optimum Methods of Cattle Breeding for Increasing Meat and Dairy Production

This Symposium was organized by Warsaw Agricultural University in cooperation with FAO and the Cattle Commission of the European Association for Animal Production (EAAP), together with the Department of Animal Production of the Ministry of Agriculture, the Institute of Zootechniques and the Polish Academy of Science's Institute of Genetics and Animal Breeding, Poland.

The meeting of the Technical Advisory Committee of the FAO International Friesian Strain Comparison Project was to be held in Warsaw in 1978 and it was decided to hold this just before the EAAP meeting in Stockholm, so that a Symposium related to the FAO Project could be attended by as many scientists and workers as possible.

Among the 14 papers presented at the Symposium were papers on cattle production and breeding in Europe, America and Africa (Botswana); the testing and comparison of various strains of Friesian cattle; beef performance of $F_1$ Friesian bulls of different strains; comparison of Bulgarian Red and Red-and-White cattle; and the level of temperate blood in dairy cattle in hot climates.

After the Symposium, participants were able to visit the sites of the FAO International Friesian Strain Comparison Project including some farms with the $F_1$ experimental heifers and cows.

The paper on beef performance of $F_1$ Friesian bulls contained data of considerable interest as regards growth rates, feed conversion, dressing percentage, fat and muscle development, and share of valuable cuts in a carcass, etc., of bulls originating from the different countries involved in the test.

In the case of dairy production, results so far available do not allow any final conclusions to be drawn, but in a forthcoming issue of this journal the first official results of this International Friesian Strain Comparison Project will be published. The first report on the Red and Red-and-White cattle breed comparison in Bulgaria was published in *World Animal Review* No. 29.

DEF

### Malaysian feedingstuffs

C. DEVENDRA, Malaysian Agricultural Research and Development Institute, PO Box 202, Serdang, Selangor, Malaysia. Hardcover. 145 pages. Price: US$ 8.00, inclusive of postage.

This publication presents the available information on the chemical composition and mineral content of feedingstuffs commonly found and used in Malaysia. A short review on literature dealing with feed composition is given at the beginning of the book.

Data are presented on the chemical composition of about 820 feedingstuffs, including the following: 38 for roots and tubers; 128 for green leaves; 128 for legumes; 158 for grasses; 131 for grains and concentrates; 141 for bulky by-products; and 92 miscellaneous feedingstuffs. In addition, data are also presented for some 490 feedingstuffs concerning macro-mineral contents (Ca, P and Mg) and for 170 concerning micro-mineral content (Na, K, Mn, Fe, Cu and Zn). Some analyses of the amino acid composition of 35 feedingstuffs are also included.

The chemical compositions of roots, tubers, legumes and a wide variety of grasses published in this book indicate that, in general, these are comparable to results reported from elsewhere in the tropics. An excellent feature of the book is the inclusion of a comprehensive set of data on tree leaves and bulky by-products, which are assuming an increasing importance in livestock feeding in the tropics.

This excellent publication provides a valuable source of information for animal scientists, extension workers, feed millers, and others interested in animal husbandry and production, not only in Malaysia but also in the whole region of Asia and the Pacific.

EKP

### A Zambian handbook of pasture and fodder crops

T.K. THORP. 1979. FAO, Rome, Italy. 139 pages.

As the author himself states, this handbook is an attempt to bring together in a compact form all the work on pasture and fodder crops carried out so far that will be useful to the farmer, extension worker, teacher and student.

The handbook is divided into 16 parts and includes chapters on pasture as a crop; pasture management; crop species suitable for the different environmental conditions of Zambia; irrigated pastures and fodder crops; dryland farming, improved planted farming, veld; improved pastures and fodder crops on small-scale farms; bush control; *dambos* (vleis); Zambesi flood plains; *barotse* sands; dry season feeding; grain legumes for use in animal feed; artificial drying of green crops; seed production and characteristics of same; improved grasses and legumes.

The handbook covers more than 25 years of pasture research in Zambia and, in view of the extensive treatment given to the wide range of pasture and fodder crops tested, many of them well adapted to other countries in the region, the publication will be of great interest to all persons concerned with tropical pasture and fodder crops development, particularly in Africa, but also in other, similar tropical areas in the world.

MDK

### New publication catalogues on bees and beekeeping

The International Bee Research Association (Hill House, Gerrards Cross, Bucks. SL9 0NR, United Kingdom) has recently issued two new catalogues. The first lists publications of the International Bee Research Association, and contains some 130 publications on various aspects of bees and beekeeping, including bee breeding, beekeeping in the tropics, bee diseases, bibliographies and visual aids in connection with bees. The second lists books selected by the Association from titles published by other organizations as being of special interest to members and others, all of which can be purchased through the IBRA.

The lists, free to Members of the Association, can be purchased at £ 0.25 or US$ 0.50. They will be of great interest to all who are in any way concerned with bees and beekeeping.

DEF

### A Chinese biogas manual

Edited by A. VAN BUREN. (Translated from the original by the Office of the Lending Group for The Propagation of Marshgas Szechuan Province, People's Republic of China.) Intermediate Technology Publications, 9 King Street, London WC2E 8HN, United Kingdom.

This excellent, well-translated and illustrated, handy-sized book of 135 pages describes very clearly and in detail how to construct a biogas pit of the kind used extensively in China for the controlled disposal of agricultural wastes (including human, animal and plant wastes) and the production of energy (methane gas) and fertilizer. Biogas production, which developed from an age-old Chinese tradition of compost making, has

evolved into a process in which materials are fermented in an airtight and watertight container in order to produce methane gas for use as fuel for motors, cooking and lighting.

In view of the growing shortage of fuel and its increasing cost this manual will be of the greatest interest to many readers in both developed and developing countries.

DEF

### Sheep breeding

Edited by G.J. Tomes, D.E. Robertson and R.J. Lightfoot. Second edition. Butterworth.

This book, in the series "Studies in the agricultural and food sciences", was produced to mark the 50th anniversary of Mureck, Western Australia's principal Agricultural College.

It covers the term "sheep breeding" in its broadest sense and includes chapters on the structure, breeds and objectives of the national sheep industries in many of the sheep-producing areas of the world. It also includes chapters on many aspects of genetic selection and breed improvement; group and cooperative breeding schemes and stud breeding; reproduction in the ewe, including synchronization of oestrus and lambing, and the culture, storage and transfer of embryos; and male reproduction and artificial insemination.

The book has numerous well-presented tables and diagrams and each chapter ends with a useful list of references.

Apart from many well-known Australian names, the authoritative contributors to the book include a wide range of nationalities. This ensures that it will be of the greatest interest and value to all those in any way concerned with sheep production and research throughout the world.

DEF

### Exotic diseases of animals — a manual for diagnosis

Service Publication (Animal Quarantine) No. 11. Second edition. Australian Government Publishing Service, Canberra.

This manual provides in a concise, well-organized form information that enables a diagnosis or presumptive diagnosis to be made of some 46 animal diseases exotic to Australia. The first part of the manual has a number of useful chapters covering such matters as procedures to be adopted by a diagnostic team in the field; equipment required by laboratory specialists going to the field; the collection, packaging and forwarding of specimens; formulae; disinfectants and disinfection; and special techniques.

Each of the chapters for the 46 exotic diseases (starting with African horse sickness and ending with Wesselsbron disease) includes comprehensive introductory sections on epizootiology, clinical signs, gross pathology and clinical differential diagnosis.

This manual, although designed specifically for use in Australia, could be of very great value to veterinary staff in other countries, especially those in Africa and Asia.

DEF

### Recent advances in animal nutrition — 1978 — 12th Nutrition Conference for Feed Manufacturers, University of Nottingham, UK, 1978.

W. Haresign and D. Lewis. Butterworth. 211 pages.

This book contains the 12 papers presented at the 12th Nutrition Conference for Feed Manufacturers.

The first session dealt with poultry nutrition, specific topics being factors affecting amino acid requirements of meat birds, protein-energy interaction in broilers and turkeys, free choice feeding of laying poultry and nutrition-disease interaction of leg weakness in poultry.

The amino-acid nutrition of the pig and the energy requirements of the growing pig were discussed at the second session together with the effects that processing and preparation of cereals for pig diets has on the efficiency of production.

One of the main topics of the Conference was the feeding of high-yielding dairy cows. The first paper given at this session, "Concentrate forage rations for high-yielding dairy cows", describes the different factors affecting the feed intake, feed utilization, health aspects and output by the dairy cows on different concentrate:forage rations. This subject was followed by a discussion on how the processing of roughages could improve the digestibility and intake of poor-quality roughages such as cereal straw.

The next two chapters indicate alternative methods of meeting the nutrient requirements of dairy cows, the first by the use of automatic feeders for the concentrate feeding of dairy cows and the second by complete diet feeding of dairy cows. The paper of the final session gives an insight into the problems and development trends in large-scale automated dairy production as experienced in a 2 500 herd unit in Sicily.

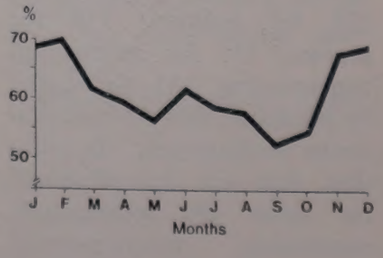
An appendix indicating the amino acid composition of a number of common feed materials completes the technical aspects of the book.

This book gives a clear and practical view of some important subjects in animal nutrition. The information is likely to be of considerable interest and value to all those working in the field of nutrition and feeding of intensively managed livestock.

EKP

### Corrigendum

*World Animal Review* 33, 1980. *Service ability and semen production of buffalo bulls.* Figures 1, 2, 3 and 4 on page 27 should be as shown here.



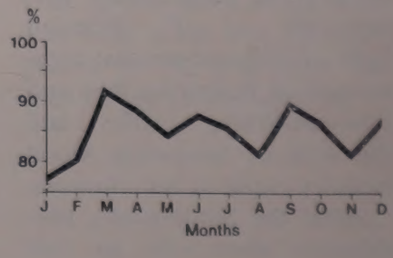




**Figure 1.** *Level of sexual activity. The percentage of the successful semen collections out of all attempted collections was used to evaluate the sexual activity of the buffalo bulls used in one year of routine work.*

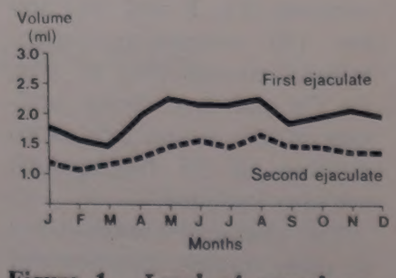



**Figure 2.** *Monthly averages of the volume of the first and second ejaculates.*

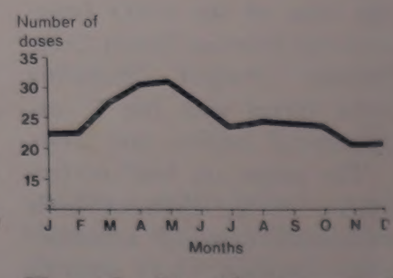



**Figure 3.** *Monthly averages of percent of collected ejaculates approved for processing (30.6 bulls; 277.5 ejaculates).*

**Figure 4.** *Number of semen doses produced from one ejaculate. Based on monthly average of 277.5 ejaculates collected from 30.6 bulls.*

# Information for contributors

We welcome letters from writers suggesting ideas and subjects for articles.

Articles for the World Animal Review should normally be of a review or report nature and should be practical and informative. However, research results, when appropriately presented, may also be acceptable. Articles on the application of new ideas, work, equipment and methods will be particularly welcome, especially when they are of direct interest and of particular value to agricultural and animal scientists in developing countries.

### Preparation of manuscripts

Manuscripts are accepted in English, French or Spanish. The Oxford English Dictionary is used as a standard for English spelling.

As far as possible the form and terminology used in articles should be straightforward and simple. Abbreviations or acronyms should be defined. Each article should be typed double-spaced on quarto paper (approximately 28 × 22 cm or 11.0 × 8.5 inches) on one side of the paper leaving margins of 5 cm (2 inches). One or two levels of headings should be sufficient for most articles.

### Length of manuscript

Articles should normally be 3 000-4 000 words in length, excluding tables and figures, with a maximum of 5 000 words for special articles. Important communications shorter than this are acceptable.

### Tables, figures, graphs, photographs

These should each be on a separate page, numbered to correspond to their points of reference in the text, well spaced for printers' instructions to be inserted clearly, and with their sources identified. Graphs and diagrams should be clearly drawn in black Indian ink on thick, smooth white paper or on thin, smooth white board. Measurements should be in the metric system.

Negatives, photographs or slides are preferred but, where these cannot be supplied, photographs should be black-and-white, clear, of full contrast and on glossy bromide paper.

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Please do not fold material. Send it suitably supported by cardboard in the envelope or package.

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